PREVALENCE OF CONGENITAL CYTOMEGALOVIRUS INFECTION AMONG NEONATES ADMITTED AT THE KENYATTA NATIONAL HOSPITAL

Principal Investigator: Dr. Esther Muthoni Ogola

Registration Number: H58/74622/2014

A dissertation submitted in partial fulfilment of the requirement for the award of the degree of Masters of Medicine in Paediatrics and Child Health of the University of Nairobi

Department of Paediatrics and Child Health,
P.O. Box 30197- 00100, Nairobi,
University of Nairobi.

2017

DECLARATION

I declare that this dissertation is my own original work and has not been published elsewhere or presented for the award of a degree in any other institution.

Signed ________________________ Date / ___/ ___ ______

Esther Muthoni Ogola, MBChB.

Senior House Officer, Department of Paediatrics and Child Health,

University of Nairobi.

This dissertation has been submitted to the University of Nairobi with our full approval as University supervisors.

Signed ____________________________ Date / ___/ ___ _____

Elizabeth Maleche Obimbo, MBChB, MMed (Paed), MPH (Epi), FPulm,

Professor of Paediatrics and Respiratory Medicine,

Department of Paediatrics and Child Health, University of Nairobi.

Signed ____________________________ Date / ___/ ____ ______

Rachel Musoke, MBChB, MMed (Paed), FNIC,

Associate Professor of Paediatrics and Newborn Medicine,

Department of Paediatrics and Child Health, University of Nairobi.

Signed ______________________________ Date / ___ / _____ _______

Ahmed Laving, MBChB, MMed (Paed)

Senior Lecturer and Paediatric Gastroenterologist,

Department of Paediatrics and Child Health, University of Nairobi

Signed _______________________ Date / ___/ ____ _______

Naomi Gachara, MBChB, MMED (Paed),

Paediatric Cardiologist,

Kenyatta National Hospital.

ACKNOWLEDGEMENTS

I would like to thank God for being with me through this process and enabling me to be able to bring it to a conclusion.

My sincere gratitude goes to my supervisors, Prof. E. Obimbo, Prof. R. Musoke, Dr. A. Laving and Dr. N. Gachara for providing me with expert guidance and support during the entire period. The knowledge I have gained from you will go a long way in shaping my career.

I would also like to thank the lecturers at the Department of Paediatrics and Child Health for your unwavering support.

My statistician, Dr. Esto Bahizire, thank you for the amazing work we have done together.

Finally, I wish to thank my family, my parents and siblings for being patient and kind with me during this period. Thank you for always believing in me and encouraging me every step of the way.

DEDICATION

I dedicate this work to my parents who have been a constant source of encouragement and my siblings who have been my rock.

TABLE OF CONTENTS

DECLARATION ii
ACKNOWLEDGEMENTS iv
DEDICATION V
TABLE OF CONTENTS VI
LIST OF TABLES IX
LIST OF FIGURES X
ABBREVIATIONS AND ACRONYMS XI
ABSTRACT 1
CHAPTER ONE. INTRODUCTION AND LITERATURE REVIEW 2
1.1 Introduction 2
1.2 Literature Review 2
1.2.1 Epidemiology 2
1.2.2 Pathophysiology 4
1.2.3 Clinical Presentation 5
1.2.4 Diagnosis 7
1.2.5 Prevention 8
1.2.6 Treatment and Follow up 8
1.2.7 Study Rationale 9
CHAPTER TWO. RESEARCH QUESTION AND STUDY OBJECTIVES 10
2.1 Research Question 10
2.2 Study Objectives 10
CHAPTER THREE. RESEARCH METHODS 11
3.1 Study Design 11
3.2 Study Site 11
3.3 Source Population 11
3.4 Study Population 11
3.5 Case Definitions 12
3.6 Mortality Outcome 13
3.7 Sample Size Determination 13
3.8 Study Tools 13

3.9 Study Procedures 14
Study Personnel 14
Subject Screening and Enrolment 14
Saliva Sample Collection and Sample Handling 15
Laboratory Procedure 15
Quality Control Measures 16
3.10 Data Management and Analysis 18
3.11 Ethical Considerations 19
CHAPTER FOUR. RESULTS 20
4.1 Description of Study Population 20
4.2 Prevalence of CMV Infection 23
4.3 Clinical Spectrum of Disease Among CMV Infected Newborns 24
4.4 Four Week Survival Outcome of CMV Infected Newborns 25
CHAPTER FIVE. DISCUSSION 28
Congenital CMV Prevalence 28
Clinical Spectrum of Illness 29
Four Week Mortality Outcome of CMV Infected Newborns 29
Limitations 30
CHAPTER SIX. CONCLUSIONS AND RECOMMENDATIONS 31
Conclusions 31
Recommendations 31
REFERENCES 32
APPENDICES 37
Appendix 1: Informed Consent Form 37
Appendix 2. Case Record Form - Enrolment 41
Appendix 3. World Health Organisation Anthropometric Growth Charts (45) ... 55
Appendix 3a: World Health Organisation Growth Chart and Head Circumference Chart for Term Male Neonates 56
Appendix 3b: World Health Organisation Growth Chart and Head Circumference Chart for Term Female Neonates 56
Appendix 4. Fentons Growth Charts and Head Circumference Charts for Preterm Neonates (49) 57
Appendix 4a: Fentons growth chart and head circumference chart for male preterms 58

Appendix 4b: Fentons growth chart and head circumference chart for preterm female neonates 58

Appendix 5. New Ballard Score Chart for Neonatal Maturity (50).......................................59

Appendix 6. Anthropometric Measurements and Physical Examination Standard Operating Procedures. 60

Appendix 6a. Standard Operating Procedures for Anthropometric Measurements 60

Appendix 7. Clinical Definitions for Clinical Spectrum of Disease.......................................62

Appendix 8. Laboratory Procedure for CMV PCR 64

Appendix 9. Normal Laboratory Values for Haemoglobin Levels..67

Appendix 10. Time Frame 69

Appendix 11. Study Budget..70

LIST OF TABLES

Table 1: Frequency of Features of Cytomegalic Inclusion Disease 6
Table 2: Symptomatic Congenital CMV Infection 12
Table 3: Infant Birth Characteristics n = 223 20
Table 4: Maternal and Socio-demographic Factors 21
Table 5: Maternal Antenatal and Delivery Characteristics 22
Table 6: Clinical Spectrum of Symptomatic Congenital CMV 24
Table 7: Four Week Mortality Outcome Among CMV Infected Newborns 25
Table 8: DNA amplification program for rotor type instruments 65

LIST OF FIGURES

Figure 1: QIAamp Min Elute Virus Spin Procedure for DNA extraction (Appendix 8).........17

Patient Follow Up.........18

Figure 4: Comparison of Survival of CMV Positive Newborns at $\leq$ 48 hours and Week 2 CMV Positive New-borns.........26

Figure 5: Comparison of Survival Between Symptomatic and Asymptomatic CMV Infected Newborn.........27

ABBREVIATIONS AND ACRONYMS

BCM	Below costal margin
CID	Cytomegalic Inclusion Disease
CMV	Cytomegalovirus.
CNS	Central Nervous System.
COX	Cyclooxygenase
CP	Cerebral Palsy
DBS	Dried Blood Spot.
DNA	Deoxyribonucleic Acid
GCV	Ganciclovir
HIG	Human Immunoglobulin.
HIV	Human Immunodeficiency Virus.
IL	Interleukin
IUGR	Intrauterine Growth Restriction.
IV	Intravenous
LBW	Low birth weight
LFT	Liver Function Test
MAS	Meconium Aspiration Syndrome
MR	Mental Retardation
NAT	Nucleic Acid Test
NEC	Necrotizing Enterocolitis
NICU	Neonatal Intensive Care Unit
PCR	Polymerase Chain Reaction.
RCT	Randomised controlled trial
RDS	Respiratory Distress Syndrome
RES	Reticuloendothelial System.
TNF	Tumour Necrosis Factor
VGCV	Valganciclovir

.

ABSTRACT

Background and Study Utility

Human Cytomegalovirus (CMV) is a leading cause of congenital infections worldwide and a leading cause of childhood hearing loss and neurodevelopmental delay. The burden of disease is in developing countries yet only few prevalence studies have been carried out in these regions. Studies in high income countries reveal prevalence ranges from 0.2 – 2.2%. The vast majority of neonates present with asymptomatic infection at birth and approximately 15% of these will develop long term neurological sequelae. Symptomatic infection occurs in 10 – 15% of the neonates with a mortality rate of 10%. A higher prevalence of congenital CMV infection has been shown in developing countries of 4 – 17.8%. Despite this, the prevalence in Kenya has not been investigated.

Study Design and Objectives

This was a short longitudinal survey aimed at determining the prevalence, clinical spectrum of disease and short term mortality of CMV among neonates admitted to the new born unit (NBU) of Kenyatta National Hospital (KNH).

Methods

We included all newborns admitted to the KNH NBU at 0 – 48 hours of life whose parents consented. A baseline history and examination were performed. Thereafter, the initial saliva sample was collected to perform a CMV Polymerase Chain Reaction (PCR) test. Patients were followed up at 2 weeks of life and a second saliva sample was then obtained from the neonates who initially tested CMV negative. A second follow up for the neonates who tested CMV positive was at 4 weeks of life. Information on morbidity during the first month was abstracted from their medical records for in-patients and by interview for illness post discharge; this was to determine the morbidity and mortality of the CMV infected newborns. Data was entered using Microsoft excel and software analysed using Stata version 11.2.

Results

We enrolled 223 newborns aged between 0-48 hours, of these, 131 (58.7%) were male; 130 (58.3%) were term with a median gestation of 37 weeks (33, 40); 118 (52.9%) were of normal weight at term with a median birth weight of 2600g (1695, 3210). Median age of the mothers was 25 years (22, 28). The most common mode of delivery was spontaneous vertex delivery at 58.3%, other modes of delivery accounted for 41.7%. Of the 223 newborns enrolled, 5 were CMV positive before 48 hours giving a prevalence of 2.2%% (95% CI 0.7 – 5.2). An additional 10, who were previously negative at birth became CMV positive by age 2 weeks, giving a 2 week prevalence of 8.6% (95% CI 4.2 – 15.3) and overall prevalence of 6.7% (95% CI 3.8 – 10.8). Of the CMV positive newborns, 5(33.3%) were symptomatic, 10(66.7%) were asymptomatic. The clinical spectrum of disease in the symptomatic newborns included; prematurity, IUGR, jaundice, hepatosplenomegaly, petechiae, pallor, thrombocytopenia, seizures, microcephaly and respiratory distress. Four of the CMV positive newborns died before age 4 weeks giving a mortality rate of 26.7%. Mortality was high among symptomatic CMV positive newborns (3 of 5, 60%) and lower among asymptomatic newborns (1 of 10, 10%) giving a relative risk for death of 8.3 (95% CI 1.2 - 58.1), P = 0.03.

Conclusions and Recommendations

The prevalence of congenital CMV among newborns hospitalised in KNH is 6.7% with majority of the neonates presenting with asymptomatic disease. Symptomatic disease occurred in one third and was associated with an eight fold increased mortality risk compared to asymptomatic infection.

We recommend that all newborns should be screened for congenital CMV due to the devastating short and long term consequences of the disease. We also recommend a study on the long-term outcome of children with congenital CMV.

CHAPTER ONE. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Human Cytomegalovirus (CMV) is an enveloped Deoxyribonucleic Acid (DNA) virus that belongs to the Betaherpesviridae sub family. CMV is species specific, has a long life cycle and establishes lifelong latency in the host monocytes and granulocytes. CMV infects and replicates in a wide variety of cells in the host, these include: epithelial cells, smooth muscle cells, fibroblasts, macrophages, dendritic cells, hepatocytes & vascular endothelial cells and is transmitted from person to person through infected body fluids. The virus can be found in blood, urine, semen, cervical secretions, saliva, breast milk and transplanted organs (1, 2).

Seroprevalence among the adult population in developing countries is 50 – 100% (3, 4) and active infection can occur in any of these three forms: Primary infection which occurs when the virus infects a CMV naïve host, endogenous infection in CMV seropositive individuals who experience reactivation from latency and exogenous reinfection in previously infected individuals who experience infection by a different strain. Infection in neonates occurs through vertical transmission leading to congenital, perinatal or postnatal infection. CMV may also be transmitted horizontally through transfusion of CMV infected blood or blood products during the neonatal period (5).

Congenital CMV (cCMV) occurs in 0.2 – 2.2% of live births globally (27). The prevalence in developing countries is however higher at 4.0 – 17.8% (6, 7, 8, 9). This is currently the most common cause of congenital infection and the leading infectious cause of brain damage and non-genetic hearing loss in children accounting for an incidence of 21% of hearing loss at birth and 25% by 4 years of age (10, 11).

cCMV is mostly asymptomatic with symptomatic infection occurring in approximately 10% of infected neonates (12). Common presentations include cranial abnormalities, thrombocytopenia, elevated transaminases, hepatosplenomegaly, jaundice, petechiae, purpura, seizures, intrauterine growth restriction (IUGR) and microcephaly. Long term neurological sequelae occur in 35 – 65% of symptomatic neonates and 7 –15% of asymptomatic neonates (13, 14).

Acquired CMV which includes; postnatal infection and that acquired through blood transfusion in neonates presents with a sepsis like syndrome in very low birth weight (VLBW) infants. Symptoms include pneumonia, hepatosplenomegaly, hepatitis, thrombocytopenia and atypical lymphocytosis (5, 17).

1.2 Literature Review

1.2.1 Epidemiology

Congenital Cytomegalovirus

A high maternal seroprevalence of CMV has been reported in developing countries. This is evidenced by research done in Thika, Kenya among pregnant women by Maingi et al that shows a maternal seroprevalence of 88.4% (4). A higher prevalence was reported in Nigeria by Ogbaini – Emovon et al at

96% (3). The increased maternal seroprevalence has been associated with the higher prevalence of congenital CMV due to increased risk of intrauterine infection.
The overall prevalence of cCMV in developing countries has been shown to be higher than that in developed countries. The prevalence is currently at 4.0 – 17.8%. Research by Bello et al in 1990 (6) and Sande et al 2007 (7) showed a prevalence of 14% and 5.4% respectively. Mwaanza et al in Zambia found a prevalence of 3.8% (8) and in Egypt, Fouhil et al, 2005 evaluated the prevalence of congenital CMV in preterm & term infants in a neonatal intensive care unit (NICU). Total prevalence was 17.8% (9).

Human Immunodeficiency Virus (HIV) infection has also been shown to be a major contributor to the high burden of CMV infection in developing countries. Slyker and Obimbo et al evaluated the incidence of CMV infection among Kenyan HIV infected infants and described an incidence of 90% in HIV exposed infants and 93% in HIV infected infants by three months of age (15). Richardson and Nduati et al showed an incidence of 80.4% among both HIV infected and exposed breastfed infants in Kenya by 1 year of life and 60.5% among formula fed HIV exposed and infected infants by one year of life (14).

The higher seroprevalence rates lead to an increased chance of either reactivation of the latent virus within a host or re infection of a seropositive host with a different strain. This increases the risk for subsequent placental or foetal infection (18).

Modes of Transmission to Neonates

Transmission of HCMV to neonates occurs vertically from mother to child. This can be; In utero through trans-placental passage to the foetus, intrapartum through infected cervical and vaginal secretions and postpartum through infected breast milk. Transmission may also occur horizontally through transfusion of infected blood and blood products (17).

In Utero Transmission

Isolation of CMV from an infant on the first three weeks after birth is evidence of in utero infection (18). A study on guinea pigs infected with guinea pig CMV was done to show evidence of transplacental infection. This was based on a number of similarities between the two host systems. Placentas obtained from pregnant guinea pigs post CMV inoculation were compared with controls that were not infected. Placental infection was noted within the first 2 weeks post inoculation & that foetal infection was dependent on placental infection and not through direct seeding (19).

Intrapartum and Postpartum Transmission

Research conducted by Dworsky et al, 1982 showed CMV secretion in CMV seropositive women. CMV was shed in breast milk in 32%, into vagina in 4%, urine in 7% & saliva in 2%. CMV was shed into breast milk most frequently between 2 & 12 weeks postpartum. Breast milk is the most common route for CMV excretion in lactating women & therefore an important source of infection (20). This is particularly in populations with high CMV seroprevalence and high breastfeeding rates. Evidence has shown that the rate of breast milk acquired CMV in neonates is directly proportional to the rate of maternal shedding of CMV in breast milk (21). A systematic review involving 26 studies was conducted by Kurath, et al, 2010 that showed a 66 – 96% detection of HCMV in breast milk of HCMV IgG positive

mothers. This review also showed that the prevalence of breast milk acquired CMV infection in infants was approximately 5.7% - 58.6% (23). Peckham et al, 1987 showed a prevalence of breastmilk acquired CMV at 12% by 3 months of life and 20% by one year of life (24). Josephon et al evaluated the transmission of CMV in VLBW infants. 27 out of 539 infants developed breastmilk acquired CMV with a 12 week incidence of 15.3%. This was determined after: Exclusion of congenital CMV, patients had no prior blood transfusion & had all received CMV nucleic acid test (NAT) positive maternal breastmilk (21). A systematic review involving 17 studies by Lanzieri, et al, 2013 estimated annual rates of breastmilk acquired CMV infection in America which varied from 2.0 – 37% (22).

Horizontal Transmission

Horizontal transmission occurs through transfusion of CMV infected blood. Prevalence of transfusion transmitted CMV infection in preterm neonates is approximately 10%. This was evident from a study done by Kim et al in 2006, after comparing rates of CMV transmission from filtered irradiated blood and non-filtered, non-irradiated blood. The overall incidence of transfusion acquired CMV infection was 4%, 2.5% in those transfused filtered irradiated blood and 10% in those transfused non filtered non irradiated blood (25).

Risk Factors

Risk factors for congenital CMV infection include a low socioeconomic status, co – infections in mother for example; malaria and HIV, NICU admittance and preterm delivery. Sande et al in The Gambia established the following risk factors: living in crowded environments, first born child and placental malarial infection (7). In Zambia, Mwaanza et al associated maternal HIV with a higher prevalence of CMV infected neonates. Babies born to HIV infected mothers had a higher chance of CMV infection at 11.4% compared to those born to HIV uninfected mothers at 2.1% (8). Fouhil et al in Egypt found a higher prevalence of CMV infection among preterm neonates admitted to a NICU at 12.2% compared to full term neonates at 5.6%. The overall prevalence of CMV infection in the NICU was 17.8% (9). Risk factors for acquired CMV in infants include high breast milk viral loads and transfusion of CMV infected blood & blood products (21).

1.2.2 Pathophysiology

After infecting the human body, the virus penetrates the cell membrane and is enclosed within a cytoplasmic vacuole. The viral nucleocapsids then enter the cell nucleus. The immediate early phase occurs where there’s restricted expression of the viral genome. This then leads to the early phase where genes are transcribed from the genome, including those of enzymes necessary for viral DNA replication. The late period then occurs, and is described as the period after viral DNA replication. This period coincides with the formation of structural proteins of the virion and the release of infectious virion. Virus assembly begins in the nucleus of the cell with formation of the capsid. Envelopment occurs in the cytoplasm and mature virions are released by lysis of infected cells or by reverse endocytosis. Spread of virions is from cell to cell. Most commonly infected cells include epithelial cells, peripheral blood mononuclear cells, cells from the CNS, endothelial cells and fibroblasts. The incubation period for CMV; which is the period from viral infection to detection of virus in the blood or

development of symptoms, has been reported to be approximately one month, with some sources reporting three to twelve weeks.

The mechanism of cell and organ damage is thought to arise from productive viral replication leading to cell lysis. It has been shown that infants with symptomatic congenital infection excrete larger amounts of virus in the first few months of life than those with asymptomatic infection. Congenitally infected infants have demonstrated chronic viral replication with viremia occurring for months to years. This chronic viral replication is likely to occur in several organs resulting in on-going cell death (26).

Inflammatory cytokines are induced by CMV contact with the human mononuclear cells. Chemokines and cytokines released include; Tumour Necrosis Factor (TNF) α, Interleukin (IL) – 6, IL -7, IL – 10, IL – 11 and Cyclooxygenase 2 (COX-2). The initial burst of inflammatory cytokines facilitates mononuclear recruitment to infection sites. Neutrophils facilitate dissemination of infection by transporting infectious virions to alternate sites of infection, whereas monocytes harbour latent infection. Cytokines such as TNF α generate signals that initiate viral replication from latency and cause persistent replication (26, 27).

Viral replication in the vascular endothelium or secondary host inflammatory response leads to vasculitis, these lead to widespread intravascular coagulopathy and damage to the involved organs. Vascular involvement may also account for the widespread disease seen in certain organs such as the CNS (26).

Inflammatory responses may be localized to areas of blood vessels in which CMV is actively replicating. This focal involvement may be the result of CMV tropism for certain cells. For example, hearing loss may be due to the result of tropism for cells located within the cochlea and eighth cranial nerve (26).

1.2.3 Clinical Presentation

Approximately 10% of the infants born with cCMV present with symptomatic disease with the majority, 90% presenting with asymptomatic but chronic infection. Symptomatic cCMV presents with acute manifestations and is known as Cytomegalic Inclusion Disease (CID). This is characterized by involvement of multiple organs, with the most commonly involved organ systems being the reticuloendothelial system (RES) and Central Nervous System (CNS) (26).

Nijman et al, 2014 and Boppana et al, 2013 defined the abnormalities most commonly identified in symptomatic cCMV as hepatomegaly, splenomegaly, petechiae, jaundice, microcephaly, IUGR, seizures, thrombocytopenia, elevated transaminases and elevated levels of conjugated bilirubin. Occurrence of microcephaly, intrauterine growth restriction (IUGR) and prematurity signifies the severity of the prenatal insult (12, 17). Mortality rate is approximately 10% in those presenting with symptomatic disease (26, 28).

Longterm neurological sequelae have been reported in more than half of patients with symptomatic cCMV. The most common complication is sensorineural hearing loss (SNHL) at approximately 60%. Other sequelae include mental retardation, cerebral palsy, seizures and chorioretinitis. Abnormal brain radiographic findings are present in approximately 50 – 70% of symptomatic cCMV at birth. Common

findings include intracranial calcifications, ventricular dilatation, cysts and lenticulostriate vasculopathy (12, 29).

Approximately 90% of newborns with congenital CMV are asymptomatic. Dollard et al, 2007 reported that approximately 13.5% of these infants develop long term neurologic sequelae, the most common being SNHL at 10 – 15% (12). Most term neonates present with asymptomatic infection because they have maternally transmitted IgG antibodies (5). 15 – 25% of preterm babies will present with a sepsis like illness including, apnoea, bradycardia, hepatosplenomegaly, distended bowel, anaemia, thrombocytopenia and abnormal hepatic function (17).

Table 1: Frequency of Features of Cytomegalic Inclusion Disease

Clinical Features **Frequency (%)**	**Mussi – Pinhata, A. Yamamoto, et al, Brazil, 2009.** **Sample size = 8047.** **Symptomatic = 87** **Age 0 – 2 weeks** **Prevalence 1.08%** (39)	**Robert Pass et al, Birmingham, 1980.** **Symptomatic = 34** **Age 0 weeks – 14 years** (38)
Preterm	-	11/32 (34)
IUGR	-	14/34 (41)
Symptoms and signs		
Petechiae	4/87 (4.6)	27/34 (79)
Purpura	2/87 (2.3)	-
Jaundice	4/87 (4.6)	20/32 (63)
Hepatosplenomegaly	4/87 (4.6)	25/34 (74)
Microcephaly	0	17/34 (50)
Seizures	1/87 (1.1)	5/23 (21.7)
Chorioretinitis	0	4/34 (12)
Sensorineural hearing loss	5/58 (8.6)	7/23 (30)
Laboratory Findings		
Thrombocytopenia	4/41 (10)	17/28 (61)
Elevated ALT/ AST	4/20 (20)	14/18 (79)
Conjugated hyperbilirubinemia	4/87 (4.6)	19/31 (61)
Radiological Findings		
Abnorml head CT scan	3/79 (3.8)	

1.2.4 Diagnosis

Diagnosis of congenital CMV is made when the virus or viral antigens are isolated from the neonate during the first two to three weeks of life. Viral shedding in an infected individual begins approximately 3 – 12 weeks after exposure (26). Detection after three weeks implies either perinatally or postnatally acquired infection. A variety of methods have been evaluated for use in the diagnosis of congenital CMV.

CMV Culture

Culture of urine or saliva has been the gold standard for identifying neonates with congenital CMV (30). There are two culture methods currently in use; the standard culture method and the rapid culture method. Standard urine culture is slow, taking two to three weeks until a report can be reported as negative. However, rapid urine culture methods have been shown to have equal sensitivity and specificity to the standard cell culture assay at 94.5% and 100% respectively. Results are available within 24 – 36 hours. Sensitivity and specificity are retained when saliva is used instead of urine. The rapid culture method is rapid, simple to perform and cheaper compared to the standard culture method (26, 31).

Nucleic Acid Amplification of DNA

Diagnosis may also be accomplished by DNA hybridization. Detection of viral nucleic acid by PCR amplification of DNA in various clinic samples, including; saliva, urine, blood, plasma, CSF, biopsy specimen has been shown to be highly sensitive and specific (26). This however varies with the sample tested. In a study conducted by Albanna et al to determine sensitivity and specificity of urine PCR, nineteen positive PCR were found from nineteen urine culture positive specimen, and none were positive from twenty urine culture negative specimen. Sensitivity was at 100% and specificity at 100% (32). Saliva PCR has comparable results to urine PCR, with liquid saliva PCR demonstrating a sensitivity of 100% and specificity of 99% while dried saliva PCR has sensitivity and specificity of 97.4% and 99.9% respectively. Saliva has an advantage over urine and blood in that it is easier to collect in newborns and the procedure is non-invasive (33).

PCR detection of CMV DNA in serum has been shown to have varying sensitivity and specificity. This depends on the nucleic acid extraction methodology used. Barbi et al, 2006 evaluated the sensitivity and specificity of CMV in serum using DBS on Guthrie cards and found a sensitivity and specificity of 100% and 99% respectively. A low sensitivity and high specificity of 30.4% and 99.9% respectively was established in PCR detection of CMV DNA in serum of more than 20000 new-borns in a multicentre study (34). One disadvantage of detection of CMV DNA in peripheral blood is that viremia may not be present in all infants with cCMV; therefore it may be impossible to detect all infected neonates (31).

Serology

Serology may also be used in the diagnosis of cCMV. This refers to the detection of CMV specific immunoglobulins. The sensitivity and specificity is however much lower as evidenced by research conducted by Albanna et al where they compared the sensitivity and specificity of IgM to the standard urine culture. Sensitivity was at 63.2% and specificity at 85% for IgM (32).

No standard method has been identified for diagnosis of perinatal CMV infection. Viral culture and DNA PCR of urine or saliva are however preferred. Serological assays present the same limitations as in diagnosing cCMV.

1.2.5 Prevention

CMV Vaccine

Transmission of CMV from the mother to the foetus can be prevented by using various methods, the most promising of which is the development of a vaccine. The vaccine consists of recombinant CMV envelope glycoprotein B with MF59 adjuvant and is currently in phase II of clinical trials. A randomized controlled trial (RCT) was performed where seronegative subjects were randomly selected into those receiving the vaccine and those receiving a placebo. 8% of those who received the vaccine developed CMV infection compared to 14% of those who received the placebo. A Kaplan-Meier analysis showed that the vaccine recipients were therefore more likely to remain uninfected compared to the placebo recipients during a 42 month period (P = 0.02). Vaccine efficacy was 50% (95% CI 7 – 73) on the basis of infection rates per 100 person years (35).

Human Immunoglobulin

Evidence has shown that CMV specific Human Immunoglobulin (HIG) is effective in reducing transmission of CMV from mother to the foetus. An RCT was conducted by Adler et al, 2007 of women randomized into those receiving HIG and those not receiving. 56% of those who did not receive HIG delivered infected infants compare with 16% of women who received prophylactic HIG. Intraamniotic HIG is also a predictor of foetal outcome as evidenced by a large multicentre case control study, with the cases being those who accepted HIG treatment. 9 of the 31 mothers receiving HIG had persistent foetal abnormalities on ultrasonography which included cerebral abnormalities, hepatic, intestinal and renal abnormalities. These neonates were however born healthy (36).

Hygiene Practices

The American College of Obstetricians and Gynaecologists guidelines recommend that physicians counsel pregnant women about preventing CMV acquisition through careful attention to hygiene. Women are encouraged to wash hands with soap and warm water after activities such as: diaper changes, feeding or bathing children, wiping child’s runny nose or drool, handling child’s toys (40).

1.2.6 Treatment and Follow up

Recommended treatment for congenital CMV is intravenous ganciclovir (IV GCV) and oral valganciclovir (VGCV). This treatment is however only recommended for symptomatic cCMV in the first 30 days of life. A phase III RCT conducted by the National Institute of allergy and Infectious Diseases Collaborative Antiviral Study Group to assess outcome of GCV treatment on symptomatic cCMV. Symptomatic newborns were enrolled and randomised into those receiving IV GCV for 6 weeks versus no treatment. GCV was shown to prevent hearing deterioration at 6 months and for those over one year of life as well as short term improvement in weight gain, head circumference and resolution of liver abnormalities. Follow up revealed GCV treated infants had less developmental delays at 6 and 12 months compared to untreated infants. The GCV treated infants were however still developmentally

behind at 6 weeks, 6 months and one year of life. Recommended dosage is 6mg/kg/dose intravenously 12 hourly for 6 weeks. Valganciclovir is the oral prodrug of ganciclovir. It is shown to have similar clinical effectiveness to IV GCV when used over 6 weeks.

New anti – CMV drugs undergoing phase IIb testing include liposomal cidofovir and the AiCuris inhibitor AIC 246 (37).

Longterm Follow Up of CMV Infected Neonates

These children also require long term follow up. These include: audiology screening according to the National Deaf Children's Society guidelines, clinical and neurodevelopmental follow up at 6 months and at least 1 year with further follow up based on clinical need and neuroimaging findings. Ophthalmological assessment is required at diagnosis to evaluate the presence of retinal scarring. Asymptomatic infants require no further examination after the one year assessment, while symptomatic infants require annual ophthalmologic assessment until 5 years to detect the presence of delayed or progressive chorioretinitis (37).

1.2.7 Study Rationale

The global burden of congenital CMV is higher in developing countries with a prevalence of 4 – 17.8% compared to developed countries with a prevalence of 0.2 – 2.2%. This has been attributed to the high adult CMV seroprevalence rate in these settings. Studies in Kenya have shown a seroprevalence of 88.4% among pregnant women in Thika and 97% among blood donors in Kenyatta National Hospital (KNH) blood transfusion unit. The high HIV prevalence has also been shown to be a risk factor with studies showing a CMV prevalence of 90 – 93% among HIV infected and exposed infants by 3 months of age.

Congenital CMV infection has devastating consequences. Despite a larger percentage of the children presenting with asymptomatic infection at birth, almost half of these will have long term neurological deficits. This poses a public health concern due to the amount of resources required for the long term follow up of these patients. Mortality rates have also been shown to be high among the symptomatic new-borns at approximately 10%.

However, development of this disease can be prevented by immunization of the mothers. Determining the prevalence of congenital CMV can influence policy makers to push for the vaccine as a top priority.

Early diagnosis and treatment within the first month of life can help prevent the long term neurological complications. Currently there is no local data on prevalence of congenital CMV infection among neonates in Kenya. Determining the prevalence of this disease will be useful to guide policy on prevention and treatment of congenital CMV in our local settings.

CHAPTER TWO. RESEARCH QUESTION AND STUDY OBJECTIVES

2.1 Research Question

What is the magnitude of congenital cytomegalovirus infection among newborns admitted to the newborn unit (NBU) of Kenyatta National Hospital (KNH)?

2.2 Study Objectives

1. To determine the prevalence of congenital cytomegalovirus infection during the first two weeks of life among neonates admitted to the newborn unit of Kenyatta National Hospital.
2. To describe the clinical spectrum of disease among cytomegalovirus infected neonates admitted to the newborn unit of Kenyatta National Hospital.
3. To determine the mortality during the first four weeks of life among cytomegalovirus infected newborns.

CHAPTER THREE. RESEARCH METHODS

3.1 Study Design

This was a hospital based short longitudinal survey.

3.2 Study Site

Patients were recruited from the NBU of KNH. This is the largest teaching and referral hospital in Kenya. It covers an area of 45.7 hectares and has a total bed capacity of 1800. The hospital caters for the low and middle income population from Nairobi and its environs as well as referrals from other hospitals in the country and the greater Eastern Africa region.

Within the KNH complex are; The College of Health Sciences (University of Nairobi medical school), the Kenya Medical Training College, Kenya Medical Research Institute and National Laboratory Services. The mission of the hospital is not only to provide health care services, but to also facilitate training and research.

The NBU of KNH has a bed capacity of 50 and has approximately 250 – 300 admissions per month from within the hospital and also from peripheral facilities across the country. Preterm neonates account for a significant proportion of the neonatal admissions. The NBU has incubators for the preterm neonates and a Neonatal Intensive Care Unit (NICU) with mechanical ventilators and Continuous Positive Airway Pressure (CPAP) machines. The unit is run by nurses, nutritionists, registrars and neonatologists.

3.3 Source Population

The source population included the neonates born in KNH or transferred from other health facilities who were admitted to the newborn unit of Kenyatta National Hospital. The study focused on neonates admitted at the NBU other than those in the postnatal wards as we wanted to capture those with symptomatic infection so as to determine the clinical spectrum of congenital CMV infection.

3.4 Study Population

Inclusion Criteria

Eligible new-borns for enrolment into the study included:

- Neonates born in KNH, referrals from other facilities, those born at home or born before arrival.
- Informed consent given from a parent.
- Age 0 -48 hours of life.

Exclusion Criteria

Neonates were excluded if they had the following:

- Baseline diagnostic sample not obtained within the first 48 hours of life.

3.5 Case Definitions

1. **Congenital cytomegalovirus infection:** was defined as the isolation of the virus or viral antigens from the neonate during the first 2 – 3 weeks of life. (44).
2. **Asymptomatic cCMV** – Diagnosis of CMV infection within the first 3 weeks of life with no detectable acute clinical abnormalities (45)
3. **Symptomatic congenital cytomegalovirus** also known as Cytomegalic Inclusion Disease: There is no standard international definition for symptomatic cCMV. We used the following definition as used in a previous study: It was defined as the diagnosis of CMV within the first 3 weeks of life in the presence of one or more of the following clinical features in the neonate as demonstrated in the following table (13,44):

Table 2: Symptomatic Congenital CMV Infection

System & Clinical Features	**Laboratory/Radiological Abnormalities**
Reticuloendothelial system Pallor Petechiae Purpura	Hb level lower than normal range for birth weight and postnatal age Thrombocytopenia Platelet count < 100x 10^9/l
Gastrointestinal System Jaundice Hepatomegaly Splenomegaly	Total Bilirubin > 1.7 – 21 μmol/l Direct Bilirubin > 1.7 – 6.8 μmol/l AST > 8 – 40 U/l ALT > 5 – 40 U/l
Central Nervous System Microcephaly Seizures Hypotonia Depressed primitive reflexes	Cranial ultrasound; Intracerebral calcification, ventricular dilatation, cortical atrophy
Respiratory System Respiratory distress	
Other Manifestations Prematurity Low birth weight Small for gestational age Failure to thrive	

For detailed clinical definitions of the above see appendix 7.

3.6 Mortality Outcome

We measured survival during the first four weeks of life. We followed up the infected neonates up to the age of four weeks and documented any deaths during that period.

3.7 Sample Size Determination

Sample size determination was based on the first specific objective which was the determination of prevalence of cCMV in neonates admitted to the NBU of KNH.

The Fischer's formula was used to compute the minimum required sample size

$N = Z^2PQ/d^2$

Where,

N = Minimum sample size.

Z = Normal standard deviation which corresponds with the 95% confidence level (1.96).

P = Estimated prevalence among neonates in an African setting. An estimated prevalence of 5% was selected based on research carried out in The Gambia by Sande et al where the prevalence of cCMV was 5.4% (7).

Q = (1-P)

d = Degree of accuracy desired set at 0.03

$N = [(1.96)^2 \times (0.05 \times 0.95)] / (0.03)^2 = 203$

We estimate that approximately 10% of patients will be lost to follow up or will die. We will cater for this by adding 10% of 203 = 20

Therefore the minimal required sample size shall be 223 babies.

3.8 Study Tools

We used 5 standardised case record forms (CRF) for data collection from the enrolled participants.

Enrolment CRF collected socio-demographic information, antenatal and perinatal information, history and examination findings of the newborn, full blood count and LFT results on admission and the case definition of CMV.

Two laboratory CRF's for saliva collection on first contact and for the repeat specimen collection at week 2. They documented the age of newborn during sample collection, date and time of saliva collection, date and time of delivery to the laboratory, quality of specimen on receipt at the laboratory and CMV results.

Follow up CRF's for week 2 and 4 that documented the history and examination findings of the new-born. The week 2 CRF also included the case definition of CMV.

3.9 Study Procedures

Study Personnel

The study personnel included: the Principal Investigator (PI), and two research assistants. Both research assistants were clinicians who had experience working in the paediatric department. The research assistants were trained on the correct technique of examining the new-borns. This included: Neonatal examination techniques, including; utilization of the New Ballard Score Chart, identification of symptoms as described in the case definition, proper sample collection and storage and how to correctly fill in the case record form.

Subject Screening and Enrolment

All potential study participants were identified on weekdays by determining the neonate's day of life. We would then approach their parents and explain the purpose and methods of the study. Written consent was sought from parents on a predesigned consent form that was handed to the guardian on first contact. The consent form explained the purpose of the study, the risks and benefits of participating in the study and also gave a brief overview of the study procedure. The investigator conducted the consent discussion and ensured that the parent fully comprehended the information on the consent form. Any questions arising regarding the study were also addressed by the investigator before the parent signed the consent form. Consent obtained from the parent was voluntary and free from coercion and was countersigned by the PI. We also obtained the telephone contacts of the mother so as to enable follow up of the patient after discharge.

Once the patient was enrolled into the study, we gave them an identification number and documented the date of the interview and date of birth. The mother was then interviewed to obtain the socio-demographic information which included: mother's age, marital status, level of education and socio economic status. We then obtained the antenatal and perinatal history.

We used the medical records to abstract information on the new-born status. This included the birth weight, sex and apgar score. We also obtained important history from these records and performed a physical examination. The general examination included the vital signs, anthropometry, and assessment for pallor, petechiae and purpura. We conducted a full examination of all systems specifically checking for the following; Abdominal, CNS, and respiratory manifestations as described in

the case definition. Refer to appendix 7. Assessment of gestational age was done using the New Ballard score. Refer to appendix 5.

The initial full blood count was documented from the medical records as all neonates had a baseline FBC on admission. Only neonates presenting with jaundice or hepatomegaly or both had LFT's done by the primary doctor, we thus also obtained this information from the medical record

Saliva Sample Collection and Sample Handling

A saliva sample was collected from all neonates enrolled into the study on first contact using sterile dry oral swabs that were labelled using the study number. The swab was removed from the packaging and inserted into the side of the cheek. Saliva was collected by using circular motions, from right to left. The swab was then placed back into the tube. Infection prevention measures were obtained during sample collection. Samples collected were packaged in plastic bags. After the last sample collection of the day, we would transport all the day's samples to the laboratory under room temperature within 24 hours from collection. Once in the laboratory, the swabs were frozen at -80^{0}C. The samples were then analysed once we obtained at least 30 samples which would be an average of 3-5 days.

Laboratory Procedure

The laboratory procedure as summarised below on figure 1 involved dissolving the sample in phosphate buffered saline (PBS) at least 12 hours before the process of DNA extraction and amplification. 200µl of this solution was then obtained for DNA extraction. There were four steps of DNA extraction which include; lysis which involved breaking up of the virus cell wall to release DNA, binding to the silica gel membrane in the spin column, washing to remove impurities and elution. The kit used for this process was known as the QIAamp Min Elute Virus Spin Kit. The final product after these four steps was pure viral nucleic acid.

The next steps involved nucleic acid amplification. This was done using the CMV Real – TM PCR kit. Nucleic acid amplification had four steps; denaturation of DNA at 95^{0}C to form single stranded DNA (ssDNA). Second step was primer annealing; this required the primers to bind to their complementary sequence in the template. It occured at temperatures of 45 – 72^{0}C; third was DNA extension. In this step DNA polymerase extended the primer by adding individual nucleotides onto the primer in a sequential manner, using the target DNA as a template. Temperatures of 72^{0}C were required for this step. The final step was termination. These steps were repeated severally. Programming was then done on the rotor gene™ 3000/6000/Q (Corbett Research Qiagen). FAM, HEX/Cy3/JOE were the filters

used to detect viral nucleic acid and the colour shown determined if the nucleic acid was from internal control(IC), the positive control, negative control or the sample. This was according to the manual.

Quality Control Measures

Internal quality control measures were through the use of NCA which was a negative control amplification. It was used as an indicator of perfect nucleic acid extraction, quality of samples and quality of the PCR. It should normally yield a negative result. It allowed to check for contamination of the reagents or artefacts that would give a false positive. Positive control of amplification was by use of the C+. The result was already known to be positive, but confirmed that there were no mistakes during the procedure. CMV PCR tests were not among the tests with external quality control at the Kenya AIDS Vaccine Initiative (KAVI) laboratory. However, KAVI laboratory obtained GCLP accreditation, as defined by Good Clinical Laboratory Practice in 2005. This was the British Association of Research Quality Assurance (BARQA-GCLP).For quality control, a defined quantity of internal control was introduced into each sample and control at the beginning of sample preparation. A NCE, NCA, C+ were required for every run to verify that the steps were carried out correctly.

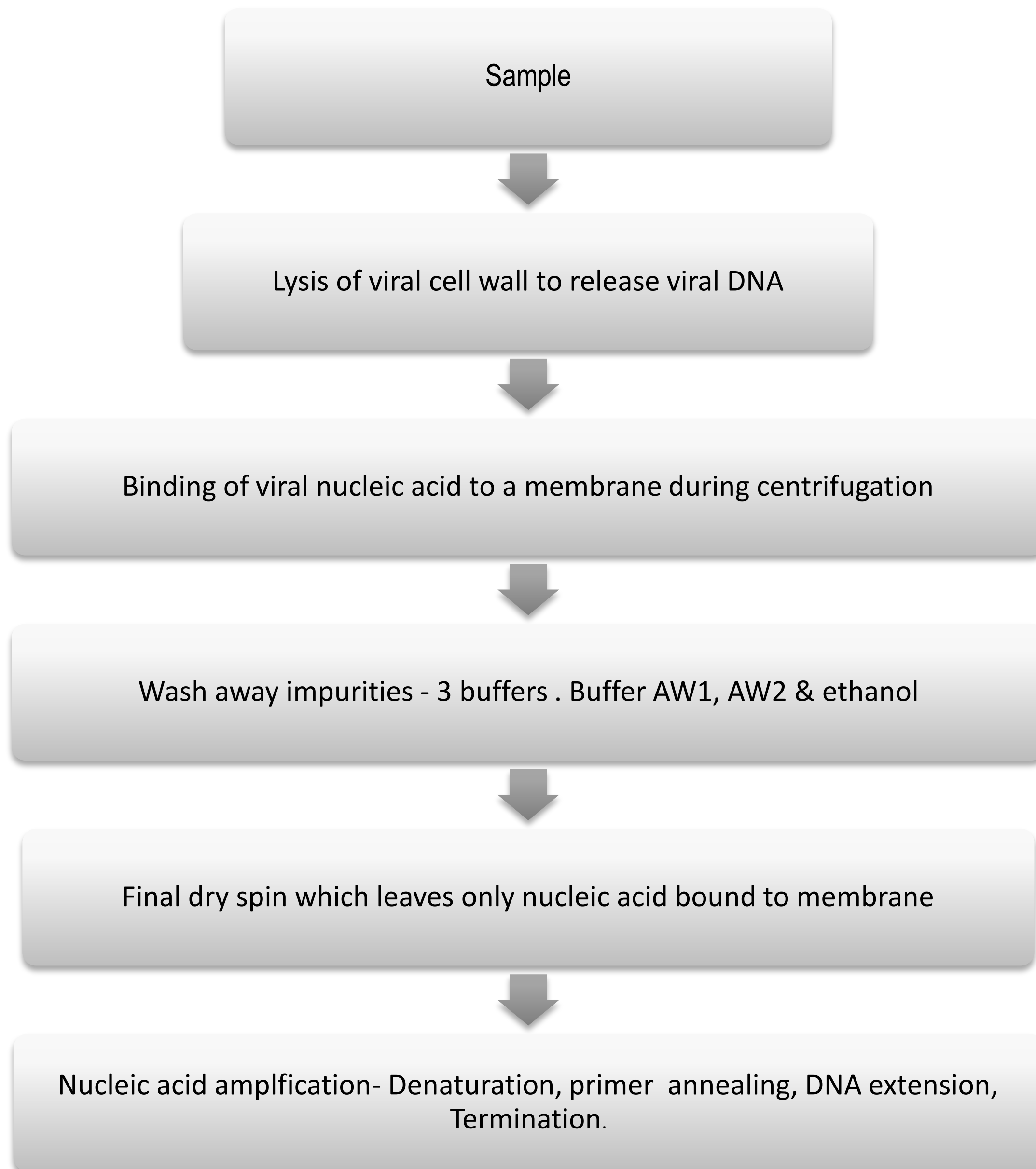


Figure 1: QIAamp Min Elute Virus Spin Procedure for DNA extraction (Appendix 8)

Patient Follow Up

The enrolled neonates who were severely ill were reviewed daily, while those who were relatively stable were next reviewed after obtaining the CMV results which would take an average of 5 days. The daily review included obtaining information from the medical records on the progress of the neonate, including; ability to breastfeed, history of fever, history of seizures and respiratory distress. We then performed a physical examination. In case of mortality, we would record the date of mortality and the circumstances surrounding the mortality. Once we obtained the CMV results, we informed the mother of the child's status. If the neonate was CMV positive, we informed the primary doctor to enable the patient to get the appropriate management. We also reviewed the CMV positive neonates daily until death or discharge.

Once the neonates were discharged, we gave an appointment for week 2 of life at the KNH new-born clinic. We also provided transport costs for the parents to bring the child back for a review. If the neonate did not come for the appointment, we called the parents to find out why, and established if the child was still alive. All neonates whether discharged or still admitted in the NBU were followed up on week 2 of life. We obtained a history as indicated above and performed a physical examination. For the neonates who tested CMV negative on day 0-48 hours of life, a repeat saliva sample was taken for CMV PCR. Once we obtained the results of the repeat CMV PCR, we informed the parents of what the results were. For all neonates who tested CMV positive either on initial contact or on week 2 of life, we gave an appointment for follow up at week 4 of life. This was to determine the morbidity and mortality of the CMV positive neonates. If the neonates did not come for the appointment, we would contact the parents to establish the condition of the neonates.

3.10 Data Management and Analysis

Data (socio demographic, clinical and biological) was recorded on the CRF by the principal investigator. Data would then be transferred onto the data set using Microsoft excel software by the principal investigator. 10% of the records were randomly checked by the statistician during data entry to ensure accuracy.

Descriptive characteristics of the study population were expressed as; categorical variables which would then be expressed as a percentage and 95% confidence interval (CI) and quantitative variables were summarized using means with standard deviation. If the distribution was not normal, we used medians and interquartile range. Proportions amongst different categories were graphically expressed as pie charts. The relationship between categorical variables would be assessed by chi square and odds ratio (95% CI) for admission data. For the follow up data which included outcome, we assessed using relative risk (95% CI) and chi square. The comparison between two means were assessed by t test or ANOVA for more than two means. For non – parametric variables we used the Mann Whitney

test to compare two variables or the Kruskal Wallis test to compare more than 2 variables (> 2 medians).

Data computation was by use of Excel programme (Microsoft Office 2010) and was analysed by Stata 11.2 (StataCorp, College Station, Texas, USA).

Survival analysis was done using time from birth to death in days as the time variable, and survival versus death during the neonatal period, Kaplan Meier Survival curves were generated.

3.11 Ethical Considerations

Permission was sought from the Kenyatta Hospital Ethics Research Committee (KHERSC) to collect and analyse data collected in the study as part of the Thesis Dissertation. Copies of this Protocol, the Informed Consent Form as well as any subsequent modifications to either document were presented to the above named committee for written approval prior to commencing the study.

The purpose of the Study was carefully explained to the Children's Parents or Guardians with a view to obtaining written consent prior to enrolling any child in the study.

No Experimental Investigations or Products were employed in this study. Non-invasive procedures were used in sample collection therefore inflicting no pain to the neonates.

Benefits that participants accrued from the study included early identification of CMV infection which is hardly tested at birth. All neonates found to be positive and symptomatic for CMV received a prescription for intravenous ganciclovir.

Strict Confidentiality was observed throughout the entire study period, held in trust by participating investigators, research staff and the study institutions. The Study Participants were given study identification numbers and no personal identification data was recorded. No Information concerning the individual study findings were released to any unauthorized third party without prior written approval of the study institution or the Ethics Research Committee.

Dissemination of our research findings will be availed to the primary health care team in the new-born unit in hopes of disseminating the knowledge gained about prevalence of CMV and its effects on our new-borns, thus encouraging healthcare providers to maintain a high index of suspicion. The study findings will also be presented to the University of Nairobi (UON) Department of Paediatrics and Child Health Academic Staff and Students in fulfilment of the requirements of the MMed Program.

CHAPTER FOUR. RESULTS

4.1 Description of Study Population

We screened 229 newborns on admission into the newborn unit in KNH between August and October 2016. Of these, 223 mothers consented to study participation and their 223 newborns were enrolled into the study.

Table 3: Infant Birth Characteristics n = 223

Patient Characteristics	**Frequency (%) or Median (Interquartile Range)**
Sex	
Male	131 (58.7)
Female	92 (41.3)
Birth weight (g)	**2600 (1695, 3210)**
<1000	5 (2.2)
1000 – 1499	25 (11.2)
1500 – 2499	75 (33.2)
2500	118 (53.4)
Gestation (weeks)	**37 (33, 40)**
< 28	6 (2.7)
28 – 31	30 (13.5)
32- 36	57 (26.0)
≥37	126 (57.8)

Clinical Characteristics of Newborns at Admission

Majority of the enrolled neonates were male (58.7%). The median gestation was 37 weeks, IQR (33, 40) and the median birth weight was 2600g, IQR (1695, 3210) as shown on table 3 above.

Maternal and Socio-demographic Characteristics

The median age of the mothers was 25 years IQR (22, 28) and were married (65.9%). The median family income was Ksh. 20000 IQR (15,000, 30000) and resided in houses made of stone walls (77.1%). (100 Kenyan shillings = 1 US dollar). This is summarised on table 4 below.

Table 4: Maternal and Socio-demographic Factors

Mother Characteristics	**Frequency (%) or** **Median (Interquartile Range)**
Mother age (years)	**25 (22, 28)**
< *20*	15 (6.7)
20 – 24	79 (35.4)
25 – 29	85 (38.1)
≥ 30	44 (19.7)
Marital status	
Married	147 (65.9)
Separated/divorced/widowed	8 (3.6)
Single	68 (30.5)
Maternal education level	
College/ University	75 (33.6)
Completed secondary	88 (39.5)
Not completed secondary	51 (22.9)
Any primary	9 (4.0)
Family Income	**20000 (15000, 30000)**
≤ 10000	35 (17.9)
11000 – 20000	63 (32.3)
21000 - ≥ 30000	97 (49.7)
Type of house	
Stone/ cement	172 (77.1)
Wood/ mabati	51 (22.9)

Antenatal and Delivery Characteristics

Attendance of at least one antenatal clinic was high among the enrolled mothers (92.8%). Majority of the mothers were HIV negative (92.3%). This was obtained both through self report and confirmation from the antenatal card. Among the enrolled newborns, the most common mode of delivery was through SVD (58.3%). The most frequent delivery complication was meconium stained liquour (19.3%).

Table 5: Maternal Antenatal and Delivery Characteristics

Variable	**Frequency (%)**
Parity	
1 – 2	150 (67.3)
3 – 4	44 (19.7)
≥5	29 (13)
ANC attendance	
Yes	207 (92.8)
No	16 (7.2)
HIV status	
Positive	17 (7.7)
Negative	206 (92.3)
Mode of delivery	
SVD	130 (58.3)
Breech/ other	3 (1.4)
Caesarean section	90 (40.3)
Delivery complications	
Prolonged rupture of membranes	6 (2.7)
Foul smelling liquour	13 (5.8)
Meconium stained liquour	43 (19.3)
Prolonged labour	25 (11.2)
Delayed second stage	11 (4.9)

4.2 Prevalence of CMV Infection

Out of the 223 new-borns enrolled into the study, 5 were positive for CMV at < 48 hours of age. The prevalence was 2.24%; 95% CI (0.7 -5.2).

At two weeks of life, 50 newborns were lost to follow up and 52 were dead. This gave a total of 116 newborns for follow up. Of these, 10 were found to be CMV positive. The prevalence was 8.6%; 95% CI (4.2 – 15.3).

The cumulative prevalence was 15/223(6.7%); 95% CI (3.8 – 10.8).

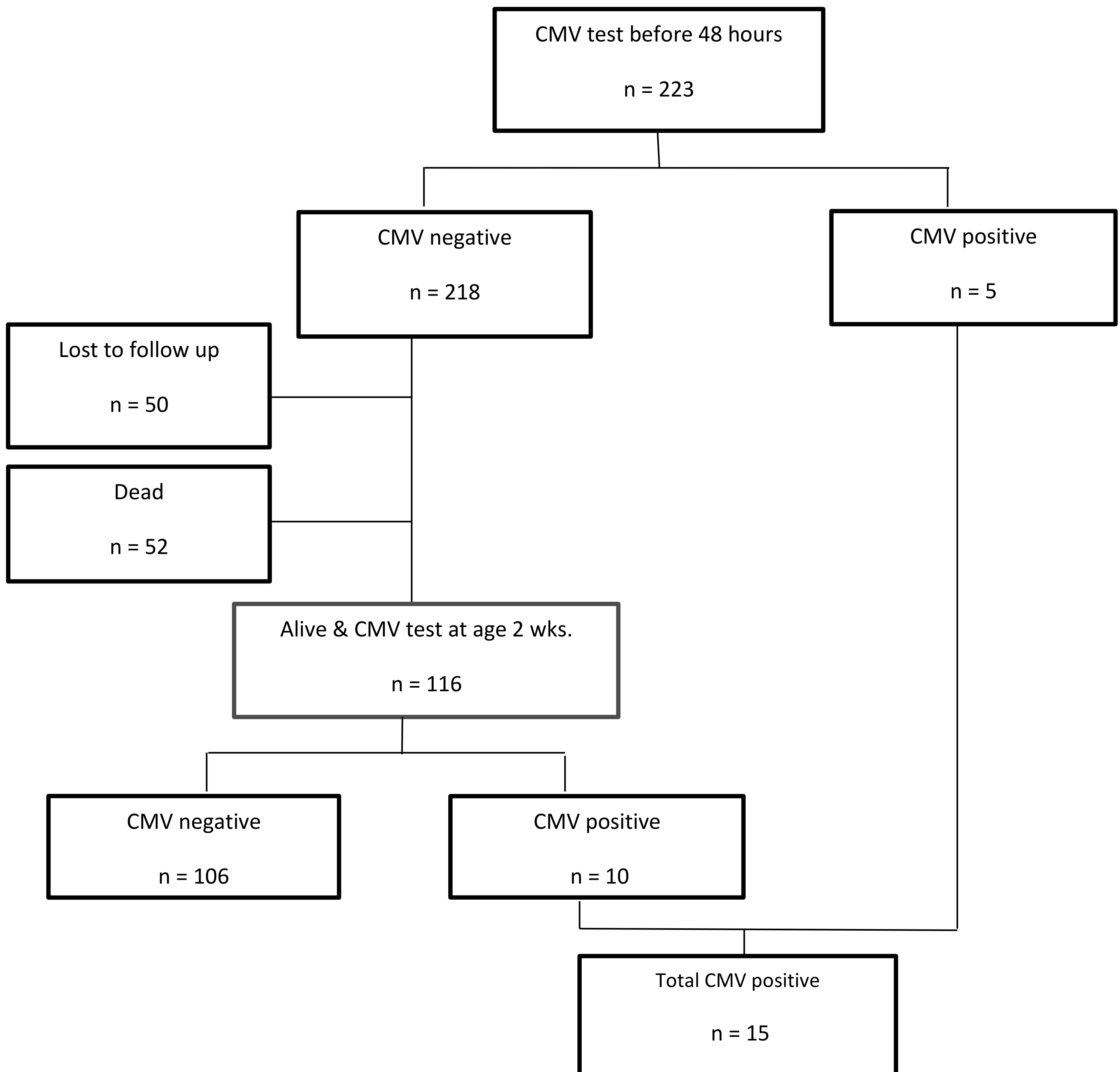


Figure 2: Flow chart showing the testing and follow up of CMV positive newborns

4.3 Clinical Spectrum of Disease Among CMV Infected Newborns

Of the fifteen CMV positive new borns, eight (53.3%) were male and seven (46.7%) were female. Twelve neonates were term, accounting for 80% and three (20%) were preterm. The median gestation was 40 weeks, IQR (37, 40) and the median birth weight was 2950g, (2470, 3200) with 11 (73.3%) being of normal birth weight and four (26.7%) being of low birth weight.

At ≤ 48 hours, out of the five CMV infected neonates, two (40%) were symptomatic, both of whom were preterm. In addition to prematurity, one of the symptomatic neonates presented with IUGR, pallor, petechiae, hepatosplenomegaly, jaundice and respiratory distress. The second symptomatic CMV positive newborn presented with prematurity and respiratory distress (Table 6).

At week two, among the ten neonates who converted from CMV negative at birth to positive by age two weeks, three (30%) were symptomatic. They had the following spectrum of clinical features: One neonate was born with IUGR (10%) but turned CMV positive by age two weeks, the second neonate presented with microcephaly, seizures and respiratory distress, accounting for 10% each and one was a preterm (10%). In total, 5/15(33.3%) were symptomatic (Table 6).

Table 6: Clinical Spectrum of Symptomatic Congenital CMV

Clinical features	CMV +ve before 48 hours. N =5 Frequency (%)	CMV +ve by 2 weeks. N = 10 Frequency (%)	Total CMV +ve. N = 15 Frequency (%)
Sex			
Male	2	6	8 (53.3)
Gestation (weeks)			
31 – 33	1 (20)	1 (10)	2 (13.3)
34 – 36	1 (20)	0 (0)	1 (6.7)
≥ 37	3 (60)	9 (90)	11 (73.3)
Birth weight (g)			
1000 - 1499	1 (20)	-	1 (6.7)
1500 – 2499	1 (20)	2 (20)	3 (20)
≥ 2500	3 (60)	8 (80)	11 (73.3)
Preterm	2 (40)	1 (10)	3 (20)
IUGR	1 (20)	1 (10)	2 (13.3)
Symptoms/ signs			
Pallor	1 (20)	-	1 (6.7)
Petechiae	1 (20)	-	1 (6.7)
Hepatosplenomegaly	1 (20)	-	1 (6.7)
Jaundice	1 (20)	-	1 (6.7)
Microcephaly	-	1 (10)	1 (6.7)
Seizures	-	1 (10)	1 (6.7)
Respiratory distress	2 (40)	1 (10)	3 (20)
Laboratory abnormalities			
Thrombocytopenia	1 (10)	-	1 (6.7)
Death	2 (40)	3 (30)	5 (33.3)

4.4 Four Week Survival Outcome of CMV Infected Newborns

Out of the 15 CMV infected neonates, both symptomatic and asymptomatic, four died, giving a total mortality of [(26.7%) 95% CI 7.8 – 55.1]. They died at a median age of five days (IQR 3.5 – 12), with individual deaths occurring at days three, four, six and eighteen. Among the five symptomatic CMV infected new borns, three died (60%). Three of the five new borns who were CMV positive within the first week died [(60%) 95% CI 14.7 – 94.7]. One of the 10 newborns who were CMV positive at week two of life died [(10%) 95% CI 0.2 – 44.5].

Table 7: Four Week Mortality Outcome Among CMV Infected Newborns

CMV Infected Neonates	No. of Babies	Number of Deaths (%)	Relative Risk of Death (95% CI)	P value
Clinical Presentation			8.3 (1.2 – 58.1)	0.03
Symptomatic	5	3 (60%)		
Asymptomatic	10	1 (10%)		
Timing of CMV Positivity			6.0 (0.82 – 44.0)	0.08
< 48 hours	5	3 (60%)		
2 weeks	10	1 (10%)		
Total	15	4(27%)		

Survival analysis revealed a 4 week survival probability of 0.58 (95% CI 0.29 – 0.78) among CMV positive newborns as demonstrated on figure 3 below.

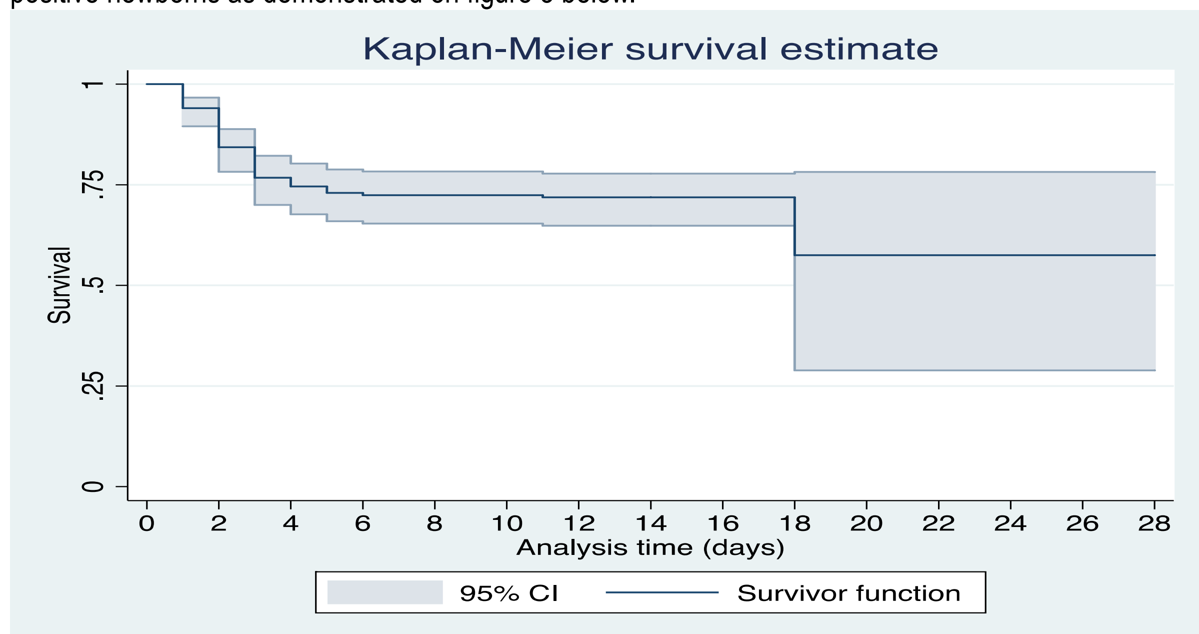


Figure 3: Four Week Survival Probability of 15 CMV Infected Newborns During The First Four

The neonates who were CMV positive at ≤ 48 hours of life had a lower survival probability of approximately 0.40 (95% CI 0.05 – 0.75) by week four of life compared to those who were CMV Among

Four Week Survival Probability Among CMV Positive at Birth Compared to Two Weeks of Life

Among birth infected newborns, there were three deaths (60%). The deaths occurred at days three, four and six, with a median age of death of four days (IQR 3, 6 days).Among week two infected babies, there was one death (10%) at age 18 days. Babies who had detectable CMV before age 48 hours had a 6 fold higher mortality than those who had CMV first detected at age 2 weeks (RR = 6.0, 95% CI 0.82 – 44.0, p = 0.08).

The neonates who were CMV positive at ≤ 48 hours of life had a lower survival probability of approximately 0.40 (95% CI 0.05 – 0.75) by week four of life compared to those who were CMV positive at two weeks with a survival probability of 0.75 (95% CI 0.13 – 0.96) as shown on (figure 4).

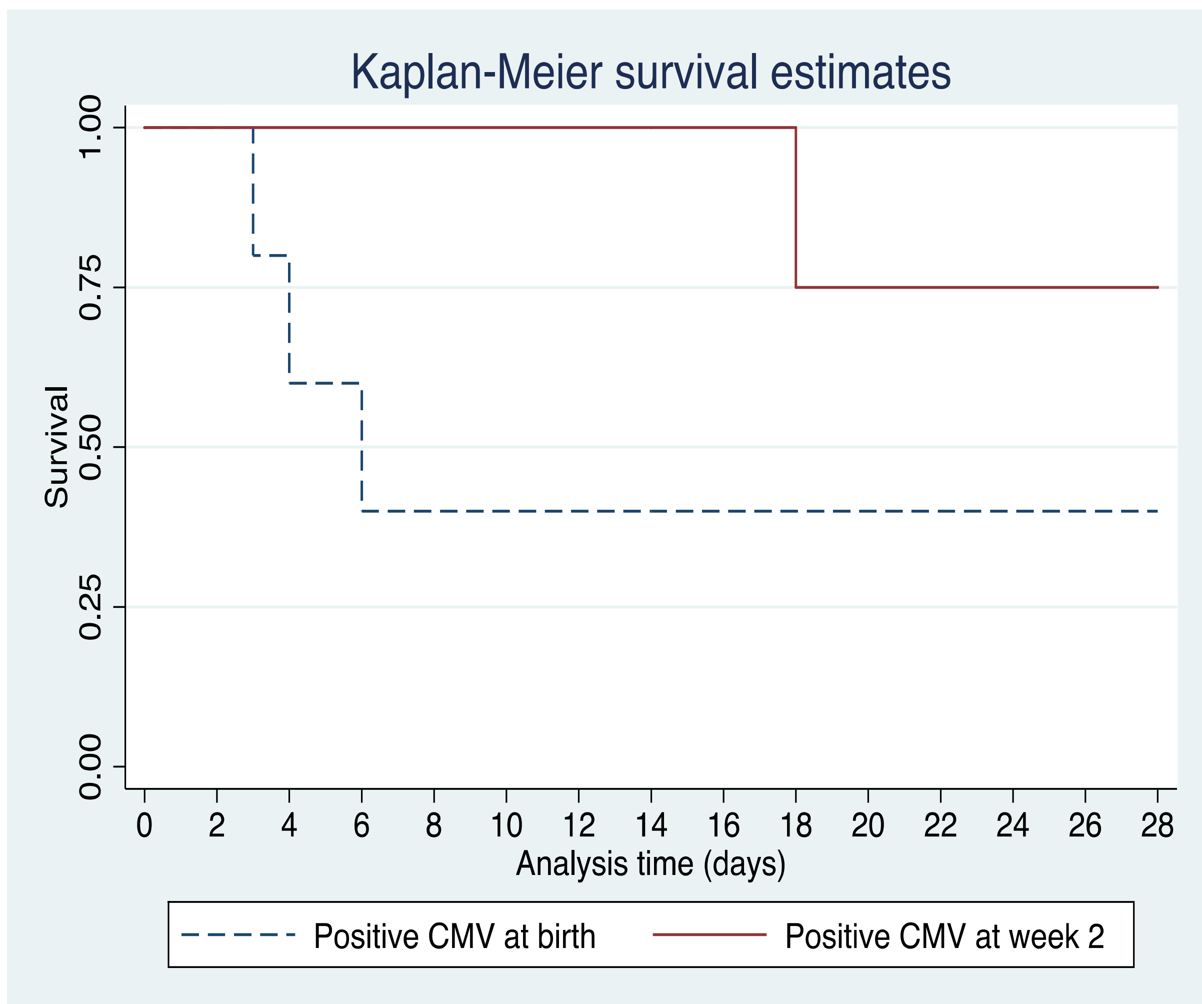


Figure 4: Comparison of Survival of CMV Positive Newborns at ≤ 48 hours and Week 2 CMV Positive New-borns.

Four Week Survival Probability Among Symptomatic CMV Infected Newborns Compared to Asymptomatic CMV Infected Newborns

Among symptomatic CMV positive babies, there were three deaths (60%). Deaths occurred at ages four, six and eighteen with a median age at death of 6 days (IQR 4, 18).Among the asymptomatic CMV positive neonates, there was one death (10%). Babies who had symptomatic CMV infection had an 8 fold higher mortality than those who had had asymptomatic infection (RR 8.3, 95% CI 1.2-58.1, p = 0.03)

The Kaplan – Meier survival analysis revealed a lower four week survival probability among the symptomatic CMV infected newborns of approximately 0.30 (95% CI 0.51 – 0.99) compared to that among the asymptomatic CMV infected newborns of approximately 0.90. This is shown on (figure 5) below.

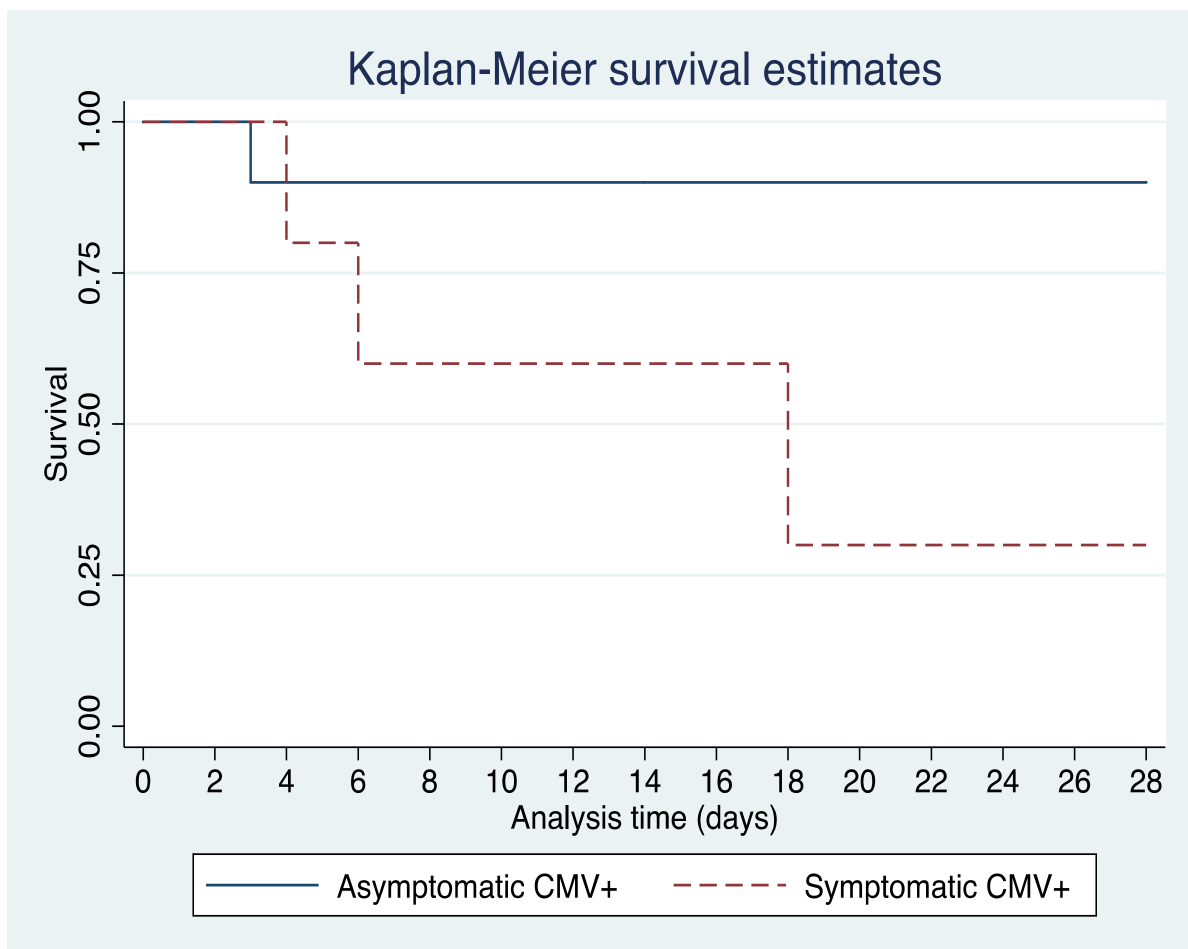


Figure 5: Comparison of Survival Between Symptomatic and Asymptomatic CMV Infected Newborn.

CHAPTER FIVE. DISCUSSION

This study sought to determine the prevalence, spectrum of clinical illness and short term outcomes of congenital cytomegalovirus infection among newborns admitted at the new born unit of Kenyatta National Hospital. To the best of our knowledge, there has been no previous local research addressing this question.

Congenital CMV Prevalence

The cumulative prevalence was 6.7% and is comparable to other African studies which have shown a prevalence of 4 -17.8%. Two African studies had a prevalence of congenital CMV that were comparable to our study results. In the Gambia, Sande et al 2007 found a prevalence of 5.4% (7) and Mwaanza et al, Zambia, demonstrated a prevalence of 3.8% (8). The congenital CMV prevalence in these two countries is comparable to our study. The similarity with the Gambia study could be due to the fact that they involved urine collection from the neonates for CMV PCR testing during the first two weeks of life. The diagnosis of congenital CMV by performing a CMV PCR test on urine is comparable to using saliva as the specimen, as done in our study, with a sensitivity and specificity of 100% each compared to 97.4% and 99.9% respectively for saliva. The maternal seroprevalence for CMV infection among mothers in the study was 100%, this is comparable to that of a study done in Kenya that shows a maternal seroprevalence of 88.4% (4). In the Zambia study, the comparable prevalence could be due to the fact that the study was carried out in Zambia's National Referral Hospital, which is similar to our study, therefore representing patients from different communities. Similar to our study, both of these studies were conducted among patients of low socioeconomic status and were limited to the population in hospitals. It is important to note that a CMV PCR test was performed on all enrolled neonates aged < 48 hours of age and a repeat CMV PCR at 2 weeks of age on all neonates who tested CMV negative on first contact.

In Egypt, a much higher prevalence of congenital CMV infection was established (17.8%). The study had some similarities to our study, it was conducted at a National Teaching and Referral Hospital and the study population was of low socioeconomic status. The sample size was much smaller as they enrolled 90 newborns and made a diagnosis of congenital CMV using both urine for CMV PCR and serological studies detecting serum IgM. The explanation could be that Egypt has a higher CMV prevalence compared to Kenya.

In Brazil, which is a middle income country, a study on the prevalence of congenital CMV was conducted at two public hospitals, one of which was a referral hospital. The prevalence of congenital CMV in this country was much lower than that in our study at 1.1%. The study had similarities to our study, which include; CMV PCR testing was done twice, at age of less than 24 hours and at week three of life, and the sample collected was either urine or saliva as they are comparable in sensitivity and specificity. Despite the similarities in the two studies, the prevalence of congenital CMV in higher income countries is much lower than that in developing countries as evidenced by this study.

It is important to note that in our study, a CMV PCR test was performed on all enrolled neonates aged < 48 hours of age and a repeat CMV PCR at two weeks of life. This was to determine if any neonates who were previously CMV negative would seroconvert within the two weeks of life within which a

diagnosis of congenital CMV could be made. We found that more neonates were positive by the second week of life (8.6%) compared to the first 48 hours of life (2.2%). A possible explanation for these results is that CMV was acquired by the neonates soon before delivery (47).

Clinical Spectrum of Illness

Of the CMV positive neonates, 10 (66.7%) were asymptomatic and 5 (33.3%) were symptomatic. Previous studies carried out in developed countries have shown that approximately 90% of CMV positive neonates present with asymptomatic infection and 10% present with symptomatic congenital CMV infection (31). In our study, we had a higher percentage of neonates with symptomatic infection. Two studies however have shown comparably higher rates of symptomatic CMV infection. Mwaanza et al, Zambia showed that six of the fifteen (40%) CMV infected newborns were symptomatic with jaundice as the most frequent presentation and petechiae as another presentation. In this study, prematurity was not considered as symptomatic (8). Fouhil et al, Egypt established that seven of the sixteen (43.8%) CMV infected newborns were symptomatic, with low birth weight as the most common presentation. The other presentations of symptomatic infection included; jaundice, hepatosplenomegaly, anaemia, thrombocytopenia, purpura and microcephaly. These were similar to our definition and findings of symptomatic congenital CMV (9). Mussi – Pinhata, et al in Brazil found a lower percentage of symptomatic infection (8.1%). Symptomatic infection had a similar definition to that in our study and the identified symptoms included: Cholestatic jaundice, petechiae, purpura, hepatosplenomegaly, seizures, cranial CT abnormalities, thrombocytopaenia and elevated liver transaminase levels (47).The higher percentage of symptomatic infection in developing countries could be due to the higher prevalence of CMV infection. As previously described, the most commonly affected systems were the reticuloendothelial system, gastrointestinal system, respiratory system and central nervous system. Due to the few neonates presenting with symptomatic CMV infection, it is difficult to give an accurate picture of the clinical spectrum of disease. The clinical spectrum of illness among the newborns presenting with symptomatic congenital CMV included; prematurity, IUGR, pallor, petechiae, thrombocytopenia, jaundice, hepatosplenomegaly and microcephaly.

On follow up at week 2 of life, 50/223 (22.4%) of neonates were lost to follow up, 52/185 (28.1%) were dead. 43 of those lost to follow up were able to give the status of the neonate over the telephone despite not appearing physically for the follow up and they were all alive and reported to be healthy.

Four Week Mortality Outcome of CMV Infected Newborns

The total mortality of CMV infected newborns in our study was four out of fifteen accounting for (26.7%). Mortality among CMV infected neonates has previously been estimated at 10% among the symptomatic neonates, this is however based on studies conducted in high income countries. Studies conducted in African countries have a shown a similarity to our study on the basis of mortality among CMV infected newborns.

Mwaanza et al, Zambia had similar results to those of our study, with mortality occurring in four of fifteen CMV positive newborns (26.7%) and was higher among the symptomatic neonates at 33.3% (8). These similarities can be explained by the fact that both studies showed a high prevalence of congenital CMV infection. A study was carried out in different hospitals across Australia by Munro S, et

al between 1999 and 2003 determining the clinical characteristics of symptomatic congenital CMV. Out of 70 identified cases, 68.2% were asymptomatic at birth or within the first month of life, out of these, mortality occurred in one neonate (1.6%) (48). This low mortality occurring among CMV infected new-borns in developed countries can be explained by the more efficient health care system compared to developed countries, therefore providing the necessary and timely treatment to the CMV infected newborns.

In our study, mortality was higher among the symptomatic newborns, occurring in three out of five symptomatic newborns (60%). One neonate with asymptomatic CMV infection died due to complications unrelated to CMV infection.There is a possibility of long-term complications among the surviving congenitally CMV infected newborns. Previous estimates have shown that on average, 35 – 65% of symptomatic neonates and 7 – 15% of asymptomatic neonates develop long term neurological sequelae with majority developing sensorineural hearing loss. Other complications include; mental retardation, cerebral palsy, seizures, chorioretinitis. They therefore require long term follow up (29).

Strengths

One of the strengths of this study was the large sample size which enables us to give good estimates of the prevalence of congenital CMV among our neonates admitted to the newborn unit in Kenyatta. There was rigorous training and close supervision of the research assistants; hence they were able to adequately examine the newborns providing adequate data. We were able to do a repeat CMV PCR at week two of life; hence we were able to get a clearer picture of the prevalence of congenital CMV by detecting the neonates who turned positive after 48 hours of life.

Limitations

The greatest limitation of this study was the large percentage of neonates who were lost to follow up or dead before two weeks of age. We were thus unable to determine their CMV status after 48 hours of age. The study was only carried out at the newborn unit, therefore leaving out the healthy newborns who were well after birth. As evidence has shown, majority of neonates with congenital CMV are asymptomatic at birth and hence we do not get a picture of the true prevalence in the community. Due to the small number of symptomatic CMV infected newborns, we were unable to get a clear picture of the clinical spectrum of disease. We were unable to verify the true cause of mortality among the symptomatic newborns, if due to CMV infection or other causes as we did not perform autopsies. Due to financial constraints we were unable to perform liver function tests on all newborns in the study; we therefore only had the liver function tests of those who presented with jaundice.

CHAPTER SIX. CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. The prevalence of congenital CMV among neonates admitted to the newborn unit of Kenyatta National Hospital was 6.7%.
2. Symptomatic congenital CMV infection commonly occurred in one third of infected newborns. The most common clinical manifestations included; prematurity, low birth weight and the haematological and respiratory systems.
3. Mortality was high among CMV infected neonates in our facility and identified risk factors were being symptomatic and detecting CMV in blood before 48 hours of age.

Recommendations

Our study provides important information to inform local guidelines on screening for and management of congenital CMV infection.

Due to the relatively high prevalence and the devastating consequences, both short and long term, we recommend that all newborns should be screened for the virus within 48 hours. This will enable proper and timely management as well as adequate long-term follow up.

A research on the long term consequences of congenital CMV should be undertaken to determine the longterm morbidity and mortality of those babies who survive the neonatal period.

REFERENCES

1. Varani S, Landini M. Cytomegalovirus-induced immunopathology and its clinical consequences.*Herpesviridae* 2011;2(6):1–14.

2. Beltran P, Cristea M. The life cycle and pathogenesis of human cytomegalovirus infection. *Expert Review of Proteomics* 2014;11(6):697-711.

3. Ogbaini-emovon E, Oduyebo O, Lofor P, Onakewhor J, Elikwu C. Seroprevalence and risk factors for cytomegalovirus infection among pregnant women in southern Nigeria. *Journal of Microbiology and Infectious Diseases* 2013;3(3):123-127.

4. Maingi Z, Nyamache A. Seroprevalence of cytomegalovirus among pregnant women in Thika, Kenya*. BMC Research Notes* 2014;7(1):1-5.

5. Kim C, Ph D. Congenital and perinatal cytomegalovirus infection. *Korean Journal of Pediatrics* 2010;53(1):15–18.

6. Bello C, Whittle H. Cytomegalovirus infection in Gambian mothers and their babies. *Journal of Clinical Pathology* 1991; 44:366-369.

7. Sande M, Kaye S, Miles D. Risk factors for and Clinical Outcome of Congenital Cytomegalovirus infection in a peri-urban West-African birth cohort. *PLoS ONE* 2007;(6):e492.

8. Mwaanza N, Chilukutu L. High rates of congenital cytomegalovirus infection linked with maternal HIV infection among neonatal admissions at a large referral center in Sub-Saharan Africa*. Clinical Infectious Diseases* 2014;58(5):728-35.

9. Fouhil D El, Ata H, Khashab A El. Congenital Cytomegalovirus Infection Among Preterm And Full-term Newborn Infants In A Neonatal Intensive Care Unit. *Egyptian Journal of Medical Microbiology* 2006;15(4):709–18.

10. Morton C, Nance W. Newborn Hearing Screening — A Silent Revolution. *N Engl J Med* 2006;354:2151–64.

11. McCollister F, Simpson L. Hearing loss and congenital symptomatic cytomegalovirus infection: A case report of multidisciplinary longitudinal assessment and intervention. *J Am Acad Audiol* 1996;7:57-62.

12. Dollard S, Grosse S, Ross D. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. *Rev. Med. Virol* 2007;17:355–63.

13. Misono S, Kathleen C, Weiss N, PH, Meei-Li Huang. Congenital cytomegalovirus infection in paediatric hearing loss. *Arch Otolaryngol Head Neck Surg* 2011;137(1):47-53.

14. Richardson BA, John-stewart G, Atkinson C, Nduati R, Ásbjörnsdóttir K, Boeckh M, et al. Vertical Cytomegalovirus Transmission From HIV-Infected Women Randomized to Formula-Feed or Breastfeed Their Infants.*Journal of Infectious Disaeses* 2015;1–7.

15. Slyker J, Lohman-Payne B, Maleche-Obimbo E, John-Stewart G. Acute cytomegalovirus infection in Kenyan HIV-infected infants. *AIDS* 2009;23(16):2173-81.

16. Manicklal S, Emery V, Lazzarotto T. The "silent" global burden of congenital cytomegalovirus. *Clinical Microbiology Reviews* 2013;26(1)86-102.

17. Nijman J, Mandemaker FS, Verboon-maciolek MA, Aitken SC, Loon AM Van, Vries LS De, et al. Genotype Distribution , Viral Load and Clinical Characteristics of Infants with Postnatal or Congenital Cytomegalovirus Infection. *PLOS ONE* 2014;9(9):1–7.

18. Bhide A, Papageorghiou A. Managing primary CMV infection in pregnancy. *BJOG* 2008;115:805-807.

19. Griffith B, Mccormick S, Fong C, Jacquelyn T, Lucia H, Goff E. The Placenta as a Site of Cytomegalovirus Infection in Guinea Pigs. *Journal of Virology* 1985;55(2):402–9.

20. Pass F. Cytomegalovirus Infection Transmission in Infancy of Breast Milk . *Pediatrics* 1983;72(3).

21. Josephson C, Caliendo A, Easley K, Knezevic A. Blood Transfusion and Breast Milk Transmission of Cytomegaloviirus in Very Low Birth Weight Infants. *JAMA Pediatr* 2014;168(11):1054–62.

22. Lanzieri A, Dollard S. Breast Milk – Acquired Cytomegalovirus Infection and Disease in VLBW and Premature Infants. *Pediatrics* 2013;131(6):1937-1945.

23. Kurath S, Resch B, Mu W, et al. Transmission of cytomegalovirus via breastmilk to the prematurely born infant: a systematic review. *Clin Microbiol Infect* 2010;16:1172-1178.

24. Peckham C, Johnson C, Ades A, Pearl K, Chin K. Early acquisition of cytomegalovirus infection. *Archives of Disease in Childhood* 1987;780–5.

25. Kim A, Lee Y, Kim K, Chu Y, Baik B, Kim E, et al. Transfusion-related Cytomegalovirus Infection Among Very Low Birth Weight Infants in an Endemic Area. *J Korean Med Sci* 2006;21:5–10.

26. Sergio Stagno. Cytomegalovirus. Remington and Klein. Infectious diseases of the foetus and newborn infant 5th edition. W. B. Saunders company;2001;7:401-413.

27. Compton T, Kurt-jones EA, Boehme KW, Belko J, Latz E, Golenbock DT, et al. Human Cytomegalovirus Activates Inflammatory Cytokine Responses via CD14 and Toll-Like Receptor 2. *Journal of Virology* 2003;77(8):4588–96.

28. Boppana S, Ross S, Fowler K. Congenital cytomegalovirus infection: Clinical outcome. *Clinical Infectious Diseases* 2013;57(S4):S178-81.

29. Boppana SB, Fowler KB, Vaid Y, Hedlund G, Stagno S, Britt WJ, et al. Neuroradiographic Findings in the Newborn Period and Long-term Outcome in Children With Symptomatic Congenital Cytomegalovirus Infection. *Pediatrics* 1997;99(3).

30. Lazzarotto T, Guerra B, Lanari M, Gabrielli L, Paola M. New advances in the diagnosis of congenital cytomegalovirus infection. *Journal of Clinical Virology* 2007;10–5.

31. Ross S, Novak Z, Boppana S. Diagnosis of cytomegalovirus infections. *Infect Disord Drug Targets* 2011;11(5):466-474.

32. Abd E, Albanna E, Saddek R, El A, Sharaf HA, Gohar MK. Diagnosis of Congenital Cytomegalovirus Infection in High Risk Neonates. *Mediterr J Hematol Infect Dis* 2013;5(1).

33. Boppana S, Ross S, et al. Saliva Polymerase-Chain-Reaction assay for cytomegalovirus screening in new-borns. *N Engl J Med* 2011;364(22):2111-2118.

34. Barbi M, Binda S, Caroppo S. Diagnosis of congenital CMV infection via dried blood spots. *Rev. Med. Virol.* 2006;16:385–92.

35. Pass R, Zhang C, Evans A, Simpson T, Huang M, et al. Vaccine Prevention of Maternal Cytomegalovirus Infection. *N Engl J Med* 2009;360(12):1191–99.

36. Adler SP, Nigro G, Pereira L. Recent Advances in the Prevention and Treatment of Congenital Cytomegalovirus Infections. *Semin Perinatol*. 2007;31:10–18.

37. Kadambari S, Williams EJ, Luck S, Grif PD, Sharland M. Early Human Development Evidence based management guidelines for the detection and treatment of congenital CMV. *Early Hum Dev* 2011;6–11.

38. Robert Pass, Sergio Stagno, Gary Myers. Outcome of Symptomatic Congenital Cytomegalovirus Infection: Results of Longoterm Longitudinal Follow up. *Pediatrics* 1980;66(5).

39. Mussi – Pinhata, A. Yamamoto, M. Brito et al. Birth Prevalence and Natural History of Congenital Cytomegalovirus Infection in a Highly Seroimmune Population. *CID* 2009;49:522 – 528.

40. Cannon M, Davis K. Washing Our Hands of The Congenital Cytomegalovirus Disease Epidemic. *BMC Public Health* 2005;5:70.

41. Akil M, Walderman A. Anemia in the Newborn Infant. In: Kliegman R, Stanton B, St. Geme J, Schor N, Behrman R, editors. *Nelson Textbook Of Pediatrics*. 19th ed. Philadelphia: Elsevier Saunders; 2011. p. 612-615.

42. Waldemar A, Carlo. The Newborn Infant. In: Kliegman R, Stanton B, St. Geme J, Schor N, Behrman R, editors. *Nelson Textbook Of Pediatrics*. 19th ed. Philadelphia: Elsevier Saunders; 2011. p. 532-541.

43. R. Harris. Children and Adolescents. In: Swash M, Glynn M, editors. *Hutchison's Clinical Methods*. 22nd ed. Philadelphia: Elsevier Saunders; 2007. p. 323-332.

44. Moore-Gillon J. Respiratory System. In: Swash M, Glynn M, editors. *Hutchison's Clinical Methods*. 22nd ed. Philadelphia: Elsevier Saunders; 2007. p. 53-69.

45. Growth Velocity Based on Weight, Lengty and Head Circumference. Geneva: World Health Organisation, 2009.

46. McLean S, Price D. Failure To Thrive. In: Kliegman R, Stanton B, St. Geme J, Schor N, Behrman R, editors. *Nelson Textbook Of Pediatrics*. 19th ed. Philadelphia: Elsevier Saunders; 2011. p. 147-149.

47. Mussi – Pinhata, Yamamoto, et al. Birth Prevalence and Natural History of Congenital Cytomegalovirus Infection in a Highly Seroimmune Population. *Clin Infect Dis.* 2009;49(4):522 -

528.

48. Munro S, Trincado D, et al.Symptomatic Infant Characteristics of Congenital Cytomegalovirus Disease in Australia*. J. Paediatr. Child Health*.2005;41:449-452.

49. Fenton, Tanis R & Kim, Jae H. A Systematic Review and Meta-analysis to Revise the Fenton Growth Chart for Preterm Infants. *BMC Pediatrics*.2013, 13:59

50. Ballard J, Khoury J, Wedig K, *et al*. New Ballard Score, Expanded to Include Extremely Premature Infants. *J Pediatrics* 1991; 119:417-423.

APPENDICES

Appendix 1: Informed Consent Form
Prevalence of Congenital CMV Infection Among Neonates Admitted To The NBU of KNH

Principal Investigator: Dr. Muthoni Ogola

TEL. NO. 0722435015

DATE __/__/____ Guardian ______________

You are invited to participate in a research study conducted by Dr. Muthoni Ogola of the University of Nairobi. Currently an MMED student in Paediatrics and Child Health.

We are asking you to take part in this study because we are interested in knowing the burden of CMV infection among new-born babies in our set up.

Your new-born was selected as a possible participant because they have been admitted to the NBU of KNH. We will ask you questions about your social and demographic factors. After this, we shall proceed to perform a basic examination on your new-born. A saliva sample will then be collected using a sterile oral swab. The results will be made known to you in 2 weeks and a prescription for antiviral medication written if your baby will test positive for the virus. Your baby will then be reviewed again at 2 and 4 weeks of life.

There are no anticipated risks to your participation in the study. Benefits however include the advantage of early diagnosis and treatment of your new-born as CMV is not routinely screened for.

Your participation in the study is completely voluntary and you may choose to stop participating at any time. Your decision not to volunteer will not influence the relationship with the researcher or study staff or with the UON either now or in future.

Any expenses you may accrue in order to avail yourself for the follow up sessions will be fully reimbursed and if any complications are identified after discharge from the hospital, we will ensure that your baby is promptly referred for further follow up at KNH.

You can stop participating in the study at any time, for any reason, if you so decide.

Confidentiality will be provided to the full extent possible by law

The research has been reviewed and approved by the Ethics Research Committee.

If you have any questions regarding the study, or the results, you may contact the Principal Investigator Dr. Muthoni Ogola on 0722435015.

If you have any concerns about this process or about your rights as a participant in the study, you may contact the Kenyatta National Hospital Ethics and Research Committee **(KNH- ESRC)** by calling **2726300 Ext. 44355**

I ______________________________ consent to participate in this study conducted by ______________________________ I have understood the nature of this project and wish to participate. My signature below indicates my consent.

Signature ………………………………… Date …………………

Participant

Signature………………………………….. Date……….

Dr. Muthoni Ogola, PI

Hali Ya Maambukizi Ya Cytomegalovirus Katika Watoto Wachanga Waliolazwa Katika Kitengo Cha Watoto Wachanga wa Hospitali Ya Taifa Ya Kenyatta.

Mpelelezi Mkuu: Dr. Muthoni Ogola

NAMBARI YA SIMU: 0722435015

Tarehe ___/___/_____ Mlezi ______________________

Wewe ni walioalikwa kushiriki katika utafiti uliofanywa na Dr. Muthoni Ogola kutoka Chuo Kikuu cha Nairobi. Sisi ni kuuliza wewe kuchukua sehemu katika utafiti huu kwa sababu sisi ni nia ya kujua mzigo wa CMV maambukizi miongoni mwa watoto wachanga katika kuweka wetu juu.

Mtoto mchanga wako alichaguliwa kama mshiriki iwezekanavyo kwa sababu amelazwa katika kitengo cha watoto wachanga wa hospitali taifa ya Kenyatta. Tutakuuliza maswali kuhusu mambo yako ya kijamii na idadi ya watu. Kisha, tutachunguza juu ya msingi wa mtoto mchanga wako. Sampuli mate kisha kukusanywa na wewe kujua matokeo katika wiki 2. Mtoto wako ataangaliwa upya tena katika wiki 2 na 4.

Hakuna hatari kwa ushiriki wako katika utafiti. Faida itakuwa utambuzi ya mapema na matibabu ya mtoto wako.

Gharama yoyote yatokanayo wakati wa kufuatilia ziara itakuwa kikamilifu fidia na kama matatizo yoyote ni kutambuliwa baada ya kutoka hospitali, mtoto wako itakuwa mara moja inajulikana kwa zaidi kufuatilia.

Ushiriki wako katika utafiti ni kwa hiari kabisa na unaweza kuchagua kuacha zinazoshiriki wakati wowote. Unaweza kuacha kushiriki katika utafiti muda wowote, kwa sababu yoyote ulivyoamua.

Usiri zitatolewa kwa kiasi kamili iwezekanavyo na sheria. Utafiti imekuwa upya na kupitishwa na kamati ya maadili ya utafiti.

Ikiwa kuna swali yoyote kuhusu utafiti huu, au matokeo, unaweza kuwasiliana na mpelelezi mkuu kwa nambari 0722435015.

Kama una wasiwasi wowote kuhusu mchakato huu au kuhusu haki zako kama mshiriki katika utafiti, unaweza kuwasiliana na Kamati ya Maadili ya Utafiti wa Hospitali ya Taifa ya Kenyatta kwa kupiga 272600 Ext. 44355.

Mimi ______________________________ Nakubali kushiriki kwa utafiti huu uliofanywa na ______________________________ Nimeelewa asili ya mradi huu. Sahihi yangu hapa chini inaonyesha idhini yangu

Sahihi ______________________________ Tarehe / / __________

Mshiriki

Sahihi ______________________________ Tarehe / / __________

Dr. Muthoni Ogola, Mpelelezi Mkuu.

Appendix 2. Case Record Form - Enrolment

Prevalence of Congenital Cytomegalovirus Infection Among Neonates Admitted To The Newborn Unit at The Kenyatta National Hospital

Principal Investigator Esther Muthoni Ogola

Study No. __________ Date of interview __/__/__________

D.O.B. __/__/________

Section A: Socio Demographic Information

1. Age of the mother (in years) ____________

2. Marital status 1. Married ( ) 2. Separated/divorced ( ) 3. Widowed ( )

   4. Single ( )

3. Maternal level of education 1. College/university ( ) 2. Completed secondary education ( ) 3. Not completed secondary education ( ) 4. Completed primary education ( ) 5. Not completed primary education ( ) 6. Nil ( ).

4. Estimated household monthly income. Ksh ______________

5. Type of house (walls) 1. Stone/cement ( ) 2. Wood ( ) 3. Mabati ( )

   4. Mud ( ).

Section B: Antenatal, Perinatal and Neonatal Information

Antenatal Information

Information from the mother and/or ANC card

1. Parity ________________
2. When was your LMP? ___/___/________
3. Gestation by dates (to the nearest week) ________________
4. When did you deliver your baby? (Dd/mm/yr) __/__/________
5. ANC attendance 1. Yes ( ) 2 No ( )
6. HIV Status
   a) Self-report 1. Negative ( ) 2. Positive ( )
   b) Confirmed from card 1. Negative ( ) 2. Positive ( )

Perinatal Information

1. Duration of labour (in hours) ________hours.

2. Mode of delivery 1. SVD ( ) 2. Breech ( ) 3. Elective C/S ( )

   4. Emergency C/S ( ) 5. Others(Specify) ______________________________________

   .

3. Delivery complications
   a) Nil
   b) Prolonged rupture of membranes 1. Yes ( ) 2. No ( )

   c) Foul smelling liquour 1. Yes ( ) 2. No ( )

   d) Meconium stained liquour 1. Yes ( ) 2. No ( )

   e) Prolonged labour 1. Yes ( ) 2. No ( )

   f) Delayed second stage 1. Yes ( ) 2. No ( )

Newborn Status

1. Birth weight _________ grams.

1. Sex of the baby M ( ) F ( )

2. Apgar Score ____________

3. Ballard score __________ Age ______weeks.

Section C: History and Examination Findings

Age at history taking and examination _________ weeks.

Part 1: Neonatal History

1. History of hotness of body Yes ( ) No ( )
2. History of respiratory distress Yes ( ) No ( )
3. History of yellowness of eyes Yes ( ) No ( )
4. History of abdominal swelling Yes ( ) No ( )

5. History of inability to breastfeed Yes ( ) No ( )
6. History of seizures Yes ( ) No ( )

Part 2a: General Examination.

1. Respiratory Rate ________/min Regular ( ) Irregular ( )
2. Heart Rate _____/min Regular ( ) Irregular ( )
3. Temperature_____ ^{0}c.
4. Gestational age ________weeks.
5. Head circumference _______cm.
6. Length __________ cm.
7. Pallor 1. Yes ( ) 2. No ( )
8. Petechiae 1. Yes ( ) 2. No ( )
9. Purpura 1. Yes ( ) 2. No ( )

Part 2b: Systemic Examination

Respiratory System

1. Respiratory rate ________ breaths/minute.
2. Nasal flaring Yes ( ) No ( )
3. Chest wall retractions Yes ( ) No ( )
4. Stridor Yes ( ) No ( )
5. Grunting Yes ( ) No ( )
6. Wheezing Yes ( ) No ( )

Conclusion: Normal respiratory findings ( ) Respiratory distress ( )

Differential diagnosis if in respiratory distress ________________ [Neonatal pneumonia, Transient Tachypnea of the Newborn (TTN), Respiratory Distress Syndrome (RDS), Meconium Aspiration Syndrome (MAS), None of the above].

Gastrointestinal System

1. Hepatomegaly Yes ( ) No ( )

   Size bcm ___cm

2. Splenomegaly Yes ( ) No ( )

   Size bcm __cm

Conclusion: Normal GIT findings ( ) Abnormal GIT findings ( )

Central Nervous System

1. Head circumference _____cm.
2. Rooting reflex present Yes ( ) No ( )
3. Suck reflex present Yes ( ) No ( )
4. Stepping reflex present Yes ( ) No ( )
5. Moro's reflex complete Yes ( ) No ( )
6. Grasp reflex present Yes ( ) No ( )
7. Hypotonia Yes ( ) No ( )
8. Hypertonia Yes ( ) No ( )

Conclusion: Normal CNS Findings ( ) Abnormal CNS findings ( )

Diagnosis if abnormal CNS findings ________________ (Prematurity, Perinatal Asphyxia, Neonatal Meningitis, None of the above).

Part 3: Laboratory Findings

1. Full blood count
   a) Haemoglobin level ________g/dl.
   b) Mean Corpuscular Volume ____ fl.
   c) Mean Corpuscular Haemoglobin ___pg.
   d) White cell count ____x10⁹/l.
   e) Neutrophils ____%
   f) Lymphocytes ____ %
   g) Platelets_____x10⁹/l
2. Liver Function Tests

a) Alanine aminotransferase _____U/L
b) Aspartate aminotransferase ____ U/L.
c) Total bilirubin _____
d) Direct bilirubin _____

Part 4: Case Definition of Symptomatic Congenital CMV (Complete this section if Saliva CMV test is positive):

- Jaundice ______
- Hepatomegaly _____
- Splenomegaly _____
- Microcephaly _____
- Hypotonia _____
- Primitive reflexes weak or absent
  a) Rooting reflex _____
  b) Sucking reflex. _____
  c) Palmar and plantar grasp ____
  d) Stepping reflex .____
  e) Moro reflex _____
- Petechiae _____
- Purpura _____
- Thrombocytopenia _____
- Anaemia _____
- Elevated transaminase levels _______
- Elevated bilirubin levels _____

Conclusion: Asymptomatic Congenital CMV ( ) Symptomatic Congenital CMV ( )

Prevalence of Congenital Cytomegalovirus Infection Among Neonates Admitted To The Newborn Unit at The Kenyatta National Hospital

Principal Investigator Esther Muthoni Ogola

Study No. ____________ Date of interview __/__/________

D.O.B. ___/ __/_ _____

Specimen Collection and Laboratory Case Record Form

Part 1: Specimen Collection

1. Date of specimen collection ____/____/________
2. Age of newborn in hours _______________
3. Quality of specimen ( )

   1 = Good 2 = Poor (Specify problem) ____________________

4. Date and time collected ______/__/______ am/pm

5. Date and time delivered to the laboratory _____/____/_____ _____am/pm

Part 2: Laboratory

1. Date and time received _/_/___ _______ am/pm.

2. Condition/ quality of specimen received ____________________________

3. Results

i. Result A ( ) 1 = Positive 2 = Negative
ii. Result B ( ) 1 = Positive 2 = Negative.

Prevalence of Congenital Cytomegalovirus Infection Among Neonates Admitted To The Newborn Unit at The Kenyatta National Hospital

Principal Investigator Esther Muthoni Ogola

Study No. ___________ Date of interview ___/___/___________

D.O.B. ___/___/_______

Follow up case record form – Week 2 of life

1. Status of baby 1 = Alive 2 = Dead.
2. If answer to question 1 was (2), kindly state:
   i. Date of death ___/___/_______
   ii. Symptoms before death ______________________________________
   iii. Clinical signs and symptoms before death (if died in hospital)

   ____

   ____

Summary assessment. Cause of death ______________________________________

3. If answer to question 1 was (1), kindly proceed to section A.

Section A: History and Examination Findings

Age at history taking and examination____________weeks.

Part 1: Neonatal History

1. History of hotness of body Yes ( ) No ( )
2. History of respiratory distress Yes ( ) No ( )
3. History of yellowness of eyes Yes ( ) No ( )
4. History of abdominal swelling Yes ( ) No ( )
5. History of inability to breastfeed Yes ( ) No ( )
6. History of seizures Yes ( ) No ( )

Part 2a: General Examination.

1. Respiratory Rate ______/min Regular ( ) Irregular ( )
2. Heart Rate _____/min Regular ( ) Irregular ( )
3. Temperature _____ ^{0}c.
4. Gestational age ______weeks.
5. Head circumference _______cm.
6. Length ___________ cm.
7. Pallor 1. Yes ( ) 2. No ( )
8. Petechiae 1. Yes ( ) 2. No ( )
9. Purpura 1. Yes ( ) 2. No ( )

Part 2b: Systemic Examination

Respiratory System

1. Respiratory rate __________breaths/minute.
2. Nasal flaring Yes ( ) No ( )
3. Chest wall retractions Yes ( ) No ( )
4. Stridor Yes ( ) No ( )
5. Grunting Yes ( ) No ( )
6. Wheezing Yes ( ) No ( )

Conclusion: Normal respiratory findings ( ) Respiratory distress ( )

Diagnosis if in respiratory distress ____________ [Neonatal sepsis (NNS), Neonatal pneumonia, Transient Tachypnoea of the New-born (TTN), Respiratory Distress Syndrome (RDS), Meconium Aspiration Syndrome (MAS), None of the above].

Gastrointestinal System

1. Hepatomegaly Yes ( ) No ( )

   Size bcm cm _____

2. Splenomegaly Yes ( ) No ( )

   Size bcm cm ____

Conclusion: Normal GIT findings ( ) Abnormal GIT findings ( )

Central Nervous System

1. Head circumference _______cm.
2. Rooting reflex present Yes ( ) No ( )
3. Suck reflex present Yes ( ) No ( )
4. Stepping reflex present Yes ( ) No ( )
5. Moro's reflex complete Yes ( ) No ( )
6. Grasp reflex present Yes ( ) No ( )
7. Hypotonia Yes ( ) No ( )
8. Hypertonia Yes ( ) No ( )

Conclusion: Normal CNS Findings ( ) Abnormal CNS findings ( )

Diagnosis if abnormal CNS findings ________________ (Prematurity, Perinatal Asphyxia, Neonatal Meningitis, None of the above).

Part 3: Laboratory Findings (If neonate has jaundice)

Full blood count if neonate is clinically pale)

a) Haemoglobin level ________g/dl.
b) Mean Corpuscular Volume _____fl.
c) Mean Corpuscular Haemoglobin ____ pg.
d) White cell count _____x10^9/l.
e) Neutrophils_____ %
f) Lymphocytes _____%
g) Platelets _____x10^9/l

Liver Function Tests

a) Alanine aminotransferase _____U/L
b) Aspartate aminotransferase ____ U/L.
c) Total bilirubin _____
d) Direct bilirubin _____

Part 4: Case Definition of Symptomatic Congenital CMV (Complete this section if Saliva CMV test is positive):

- Jaundice ______
- Hepatomegaly _____
- Splenomegaly _____
- Microcephaly _____
- Hypotonia _____
- Primitive reflexes weak or absent
    a) Rooting reflex _____
    b) Sucking reflex. _____
    c) Palmar and plantar grasp ____
    d) Stepping reflex . ____
    e) Moro reflex _____
- Petechiae ______
- Purpura ______
- Thrombocytopenia _____
- Anaemia ______
- Elevated transaminase levels _______
- Elevated bilirubin levels ______

Conclusion: Asymptomatic Congenital CMV ( ) Symptomatic Congenital CMV ( )

Prevalence of Congenital Cytomegalovirus Infection Among Neonates Admitted To The Newborn Unit at The Kenyatta National Hospital

Principal Investigator Esther Muthoni Ogola

Study No. ____________ Date of interview ___/___/________

D.O.B. ___/___/________

Specimen Collection – Week 2 of life

a) Date of specimen collection ___/___/________
b) Age of newborn in Weeks ________________
c) Quality of specimen ( )

1 = Good 2 = Poor (Specify problem) ______________________________

d) Date and time collected ___/___/________ ________am/pm

e) Date and time delivered to the laboratory ___/___/________ ________am/pm

Laboratory

1. Date and time received ___/___/________ ________am/pm

2. Condition/ quality of specimen received __________________________

3. Results

iii. Result A ( ) 1 = Positive 2 = Negative
iv. Result B ( ) 1 = Positive 2 = Negative.

Prevalence of Congenital Cytomegalovirus Infection Among

Neonates Admitted To The Newborn Unit at The Kenyatta National Hospital

Principal Investigator Esther Muthoni Ogola

Study No. ____________ Date of interview ___/___/__________

D.O.B. ___/___/________

Follow up form – Week 4 of life

1. Status of baby 1 = Alive 2 = Dead.
2. If answer to question 1 was (2), kindly state:
   i. Date of death ___/___/________
   ii. Circumstances surrounding death
   iii. Symptoms before death ____

   ____
   iv. Clinical signs and symptoms before death (if died in hospital)

   ____

   ____

Summary assessment. Cause of death ____

3. If answer to question 1 was (1), kindly proceed to section A.

Section A: History and Examination Findings

Age at history taking and examination __________ weeks.

Part 1: Neonatal History

1. History of hotness of body Yes ( ) No ( )
2. History of respiratory distress Yes ( ) No ( )
3. History of yellowness of eyes Yes ( ) No ( )
4. History of abdominal swelling Yes ( ) No ( )
5. History of inability to breastfeed Yes ( ) No ( )

6. History of seizures Yes ( ) No ( )

Part 2a: General Examination.

1. Respiratory Rate________/min Regular ( ) Irregular ( )
2. Heart Rate _______/min Regular ( ) Irregular ( )
3. Temperature______ ^{0}c.
4. Gestational age _________weeks.
5. Head circumference __________cm.
6. Length ____________cm.
7. Pallor 1. Yes ( ) 2. No ( )
8. Petechiae 1. Yes ( ) 2. No ( )
9. Purpura 1. Yes ( ) 2. No ( )

Part 2b: Systemic Examination

Respiratory System

1. Respiratory rate ___________breaths/minute.
2. Nasal flaring Yes ( ) No ( )
3. Chest wall retractions Yes ( ) No ( )
4. Stridor Yes ( ) No ( )
5. Grunting Yes ( ) No ( )
6. Wheezing Yes ( ) No ( )

Conclusion: Normal respiratory findings ( ) Respiratory distress ( )

Diagnosis if in respiratory distress ________________[Neonatal sepsis (NNS), Neonatal pneumonia, Transient Tachypnea of the Newborn (TTN), Respiratory Distress Syndrome (RDS), Meconium Aspiration Syndrome (MAS), None of the above].

Gastrointestinal System

1. Hepatomegaly Yes ( ) No ( )

   Size bcm ____cm

2. Splenomegaly Yes ( ) No ( )

   Size bcm __cm

Conclusion: Normal GIT findings ( ) Abnormal GIT findings ( )

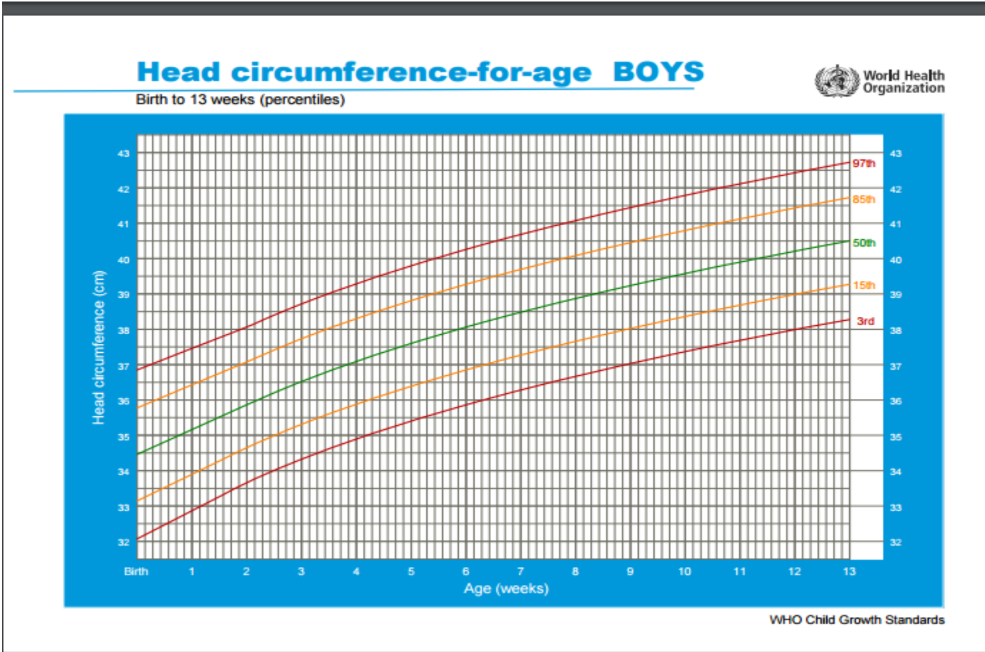
Central Nervous System

1. Head circumference ______cm.
2. Rooting reflex present Yes ( ) No ( )
3. Suck reflex present Yes ( ) No ( )
4. Stepping reflex present Yes ( ) No ( )
5. Moro's reflex complete Yes ( ) No ( )
6. Grasp reflex present Yes ( ) No ( )
7. Hypotonia Yes ( ) No ( )
8. Hypertonia Yes ( ) No ( )

Conclusion: Normal CNS Findings ( ) Abnormal CNS findings ( )

Diagnosis if abnormal CNS findings__________________(Prematurity, Perinatal Asphyxia, Neonatal Meningitis,None of

Appendix 3. World Health Organisation Anthropometric Growth Charts (45)



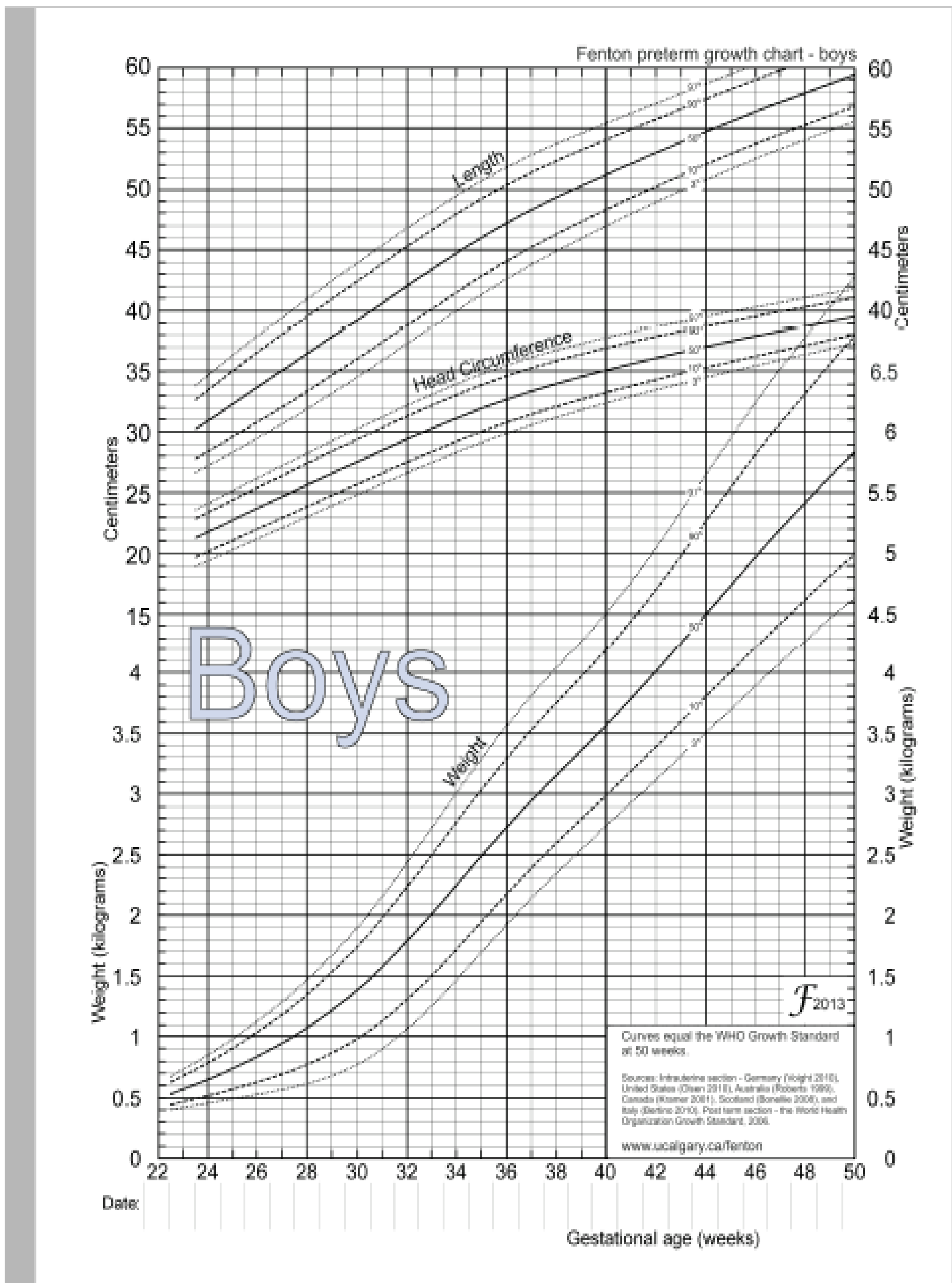
Appendix 3a: World Health Organisation Growth Chart and Head Circumference Chart for Term Male Neonates

Head circumference-for-age GIRLS

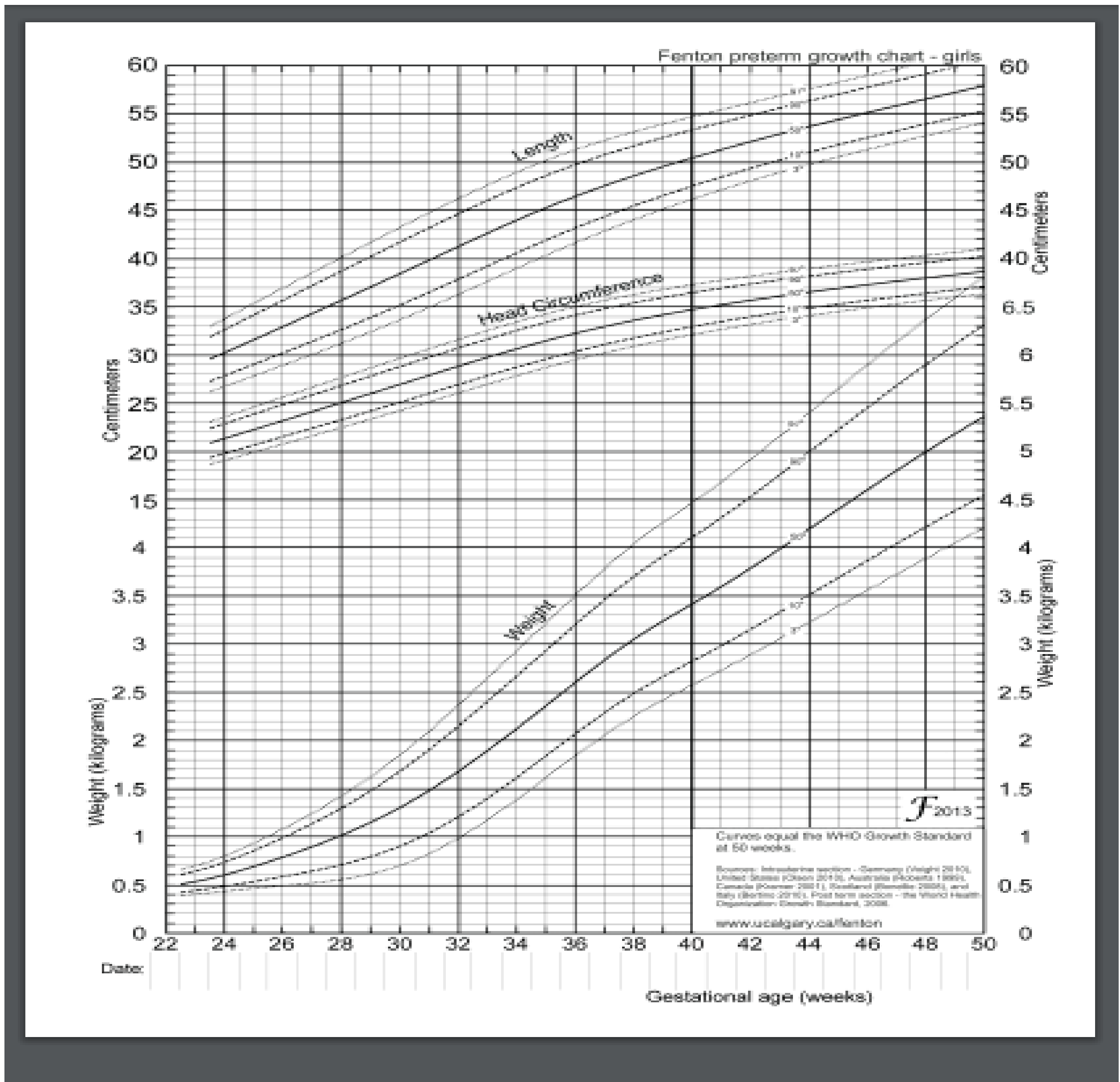
Birth to 13 weeks (percentiles)

WHO Child Growth Standards

Appendix 4. Fentons Growth Charts and Head Circumference Charts for Preterm Neonates (49)



Appendix 4a: Fentons growth chart and head circumference chart for male preterms



Appendix 5. New Ballard Score Chart for Neonatal Maturity (50)

NEUROMUSCULAR MATURITY

NEUROMUSCULAR MATURITY SIGN	SCORE -1	0	1	2	3	4	5	RECORD SCORE HERE
POSTURE								
SQUARE WINDOW (Wrist)	>90°	90°	60°	45°	30°	0°		
ARM RECOIL		180°	140–180°	110–140°	90–110°	<90°		
POPLITEAL ANGLE	180°	160°	140°	120°	100°	90°	<90°	
SCARF SIGN								
HEEL TO EAR								
							TOTAL NEUROMUSCULAR MATURITY SCORE	

PHYSICAL MATURITY

PHYSICAL MATURITY SIGN	SCORE -1	0	1	2	3	4	5	RECORD SCORE HERE
SKIN	sticky friable transparent	gelatinous red translucent	smooth pink visible veins	superficial peeling &/or rash, few veins	cracking pale areas rare veins	parchment deep cracking no vessels	leathery cracked wrinkled	
LANUGO	none	sparse	abundant	thinning	bald areas	mostly bald		
PLANTAR SURFACE	heel-toe 40–50 mm: -1 < 40 mm: -2	>50 mm no crease	faint red marks	anterior transverse crease only	creases ant. 2/3	creases over entire sole		
BREAST	imperceptible	barely perceptible	flat areola no bud	stippled areola 1–2 mm bud	raised areola 3–4 mm bud	full areola 5–10 mm bud		
EYE / EAR	lids fused loosely: -1 tightly: -2	lids open pinna flat stays folded	sl. curved pinna; soft; slow recoil	well-curved pinna; soft but ready recoil	formed & firm instant recoil	thick cartilage ear stiff		
GENITALS (Male)	scrotum flat, smooth	scrotum empty faint rugae	testes in upper canal rare rugae	testes descending few rugae	testes down good rugae	testes pendulous deep rugae		
GENITALS (Female)	clitoris prominent & labia flat	prominent clitoris & small labia minora	prominent clitoris & enlarging minora	majora & minora equally prominent	majora large minora small	majora cover clitoris & minora		
							TOTAL PHYSICAL MATURITY SCORE	

SCORE

Neuromuscular ______

Physical ______

Total ______

MATURITY RATING

SCORE	WEEKS
-10	20
-5	22
0	24
5	26
10	28
15	30
20	32
25	34
30	36
35	38
40	40
45	42
50	44

GESTATIONAL AGE (weeks)

By dates ______

By ultrasound ______

By exam ______

Reference

Ballard JL, Khoury JC, Wedig K, et al: New Ballard Score, expanded to include extremely premature infants. J Pediatr 1991; 119:417-423. Reprinted by permission of Dr Ballard and Mosby—Year Book, Inc.

Appendix 6. Anthropometric Measurements and Physical Examination Standard Operating Procedures.

Appendix 6a. Standard Operating Procedures for Anthropometric Measurements

Weight was measured using a digital infant scale. The scale was covered with paper and activated by turning it on such that it displayed zero on the display panel. The baby would be undressed and placed on the tray of the scale. The weight would then be recorded to the nearest 0.1kg as it appeared on the display panel.

Length assured using a tape measure. Neonate was placed in the supine position with an assistant gently holding down the neonates legs at the knees. The tape measure was placed on the right side of the neonate and the length measured from the top of the head to the heel. The length was then recorded to the nearest 0.1cm.

Head Circumference This was determined by measuring the occipitofrontal circumference. This was the circumference from the occiput to the supraorbital ridges.

Appendix 6b: Standard Operating Procedures for Evaluation of Neonatal Reflexes

Rooting reflex we used our index finger to touch the neonates cheek and observed if the neonate would turn his head towards the stimulus.

Sucking reflex. We stimulated the roof of the neonate's mouth with our index finger and observed if the neonate instinctively began to suck.

Palmar and plantar grasp. We placed an index finger across the child's palm or plantar aspect of foot and tested for flexion and grasping of the finger by the neonate.

Stepping reflex. We held the baby upright with the feet placed on a firm surface. As the foot pressed down, the other leg flexed at the hip and knee in a stepping movement.

Moro reflex. This was performed as the last examination so as not to agitate the neonate. The baby's body was supported by one hand and the head with the other. The hand holding the head was lowered a few centimetres, allowing the baby's head to drop back. In a positive response, the baby would abduct and extend the arms, then flex them.

Tone. We first observe the posture of the neonate. Normal posture was in flexion. We then assessed the upper extremity tone using a passive range of motion. This was done by rotating each limb at the shoulder, elbow and wrist and feeling the resistance and range of motion. Too little resistance reflected hypotonia while too much reflected hypertonia. Lower extremity tone was assessed by assessing for passive range of motion by flexing the hips, then abducting and adducting the hips, knees and ankles.

Appendix 6c: Standard Operating Procedures for Evaluation of Abdominal Organomegaly

Hepatomegaly was elicited by ballottement. We began at the right subcostal region as we moved lower if we felt any resistance. We moved lower until the resistance disappeared. The lower edge of the liver

was clarified by percussion. This was marked with a pen and a tape measure used to measure in centimetres the size of the liver palpable below the costal margin.

Splenomegaly was elicited using the balloting method of palpation. The examiner's dominant hand was placed at the umbilical region and palpated diagonally towards the left subcostal margin. If a mass was palpable, whose upper border could not be felt or palpated a notch, this was most likely the spleen. We clarified the lower border by percussion and marked the lowest point using a pen. Then used a tape measure to assess this length from the left costal margin.

Appendix 7. Clinical Definitions for Clinical Spectrum of Disease
Abdominal symptoms

Jaundice which was defined as the yellow colouration of the sclera and/or skin in the neonate due to increased bilirubin levels. Grading was based on the grading scheme of Kramer which is based on the clinical extent. Grade 2 – involving chest and upper abdomen and/or back. Grade 3 – involves abdomen below umbilicus to the knees. Grade 4 involves the legs below the knees and/or upper and lower arms. Grade 5 – involves hands and/or feet (42).

Hepatomegaly was defined as a liver palpable > 2 cm below the right costal margin (42).

Elevated transaminase levels were defined as alanine transaminase levels and aspartate transaminase levels above the reference range in the KNH laboratory. The normal ranges were: Alanine Aminotransferase – 5-40U/L and Aspartate Aminotransferase – 8-40U/L

Elevated bilirubin levels. Bilirubin levels above the normal reference range for the KNH laboratory. The normal ranges were: Total Bilirubin – 1.7 – 21 μmol/l and direct Bilirubin – 1.7 – 6.8μmol/l.

Splenomegaly was defined as a spleen palpable > 2 cm below the left costal margin (42).

CNS manifestations

Microcephaly was defined as a head circumference that is ≤ 2 standard deviations (SD) below the mean compared to age and gender- matched population - based samples or less than the 5th percentile for age and sex. (42)

Seizures were defined as a transient occurrence of signs and/or symptoms resulting from abnormal excessive or synchronous neuronal activity in the brain. In neonates mostly present as subtle seizures, clonic, tonic, spasms or myoclonic seizures.

Hypotonia was defined as a subjective decrease of resistance to passive range of motion in a term newborn.

Depressed primitive reflexes (43).

a) **Rooting reflex** was defined as the response of the child to a touch on the cheek whereby the neonate would turn his head towards the stimulus.
b) **Sucking reflex**. This was where a neonate instinctively sucked anything stimulating the roof of their mouth.
c) **Palmar and plantar grasp** were defined as flexion and grasping of a finger placed across the child's palm or plantar aspect of foot.
d) **Stepping reflex**. This was when the baby was held upright and the feet placed on a firm surface. As the foot pressed down, the other leg flexed at the hip and knee in a stepping movement.
e) **Moro reflex**. This was positive when a baby abducted and extended the arms, then flexed them when a hand held to support the baby's head was lowered a few centimetres and the head allowed to drop back.

Haematological manifestations (41)

Petechiae were defined as minute (<2mm), pinpoint, non –raised, purplish red spot containing blood that appears in the skin or mucous membrane.

Purpura were defined as a haemorrhagic state characterized by patches of purplish discolouration (>2mm) resulting from extravasation of blood into the skin and mucous membrane.

Thrombocytopenia was defined as a reduction in platelet count platelet count below $100x10^9/l$ which corresponded to values below the 5th percentile.

Anaemia was defined as a haemoglobin value lower than the normal range of haemoglobin for birth weight and postnatal age.

Respiratory manifestations (44)

Respiratory distress was defined as signs and symptoms of abnormal respiratory pattern. It included any of; nasal flaring, tachypnoea, chest wall retractions, stridor, grunting, wheezing and dyspnoea.

Tachypnoea in a neonate was defined as a respiratory rate of more than 60 breaths/ minute.

Grunting was defined as an abnormal sound heard during laboured exhalation created by rhythmic closure of the glottis at the end of expiration.

Wheeze was defined as a musical sound on auscultation associated with airway narrowing and was particularly heard on expiration.

Stridor was characterized by monophonic audible breath sounds that originated from the extra thoracic airways.

Other Manifestations

Preterm neonates were defined as those neonates born alive before 37 completed weeks of pregnancy (45).

- **Extremely preterm** were those neonates born at a gestation of < 28 weeks.
- **Very preterm** were neonates born at a gestation between 28 to < 32 weeks.
- **Late preterm** were defined as the neonates born at a gestation of 32 to < 37 weeks.

Low birth weight defined as a weight at birth of less than 2500g irrespective of gestational age.

- **Extremely low birth weights** were those neonates born with a weight of < 1000g.
- **Very low birth weights** were those neonates born with a birth weight of 1000 – 1499g.
- **Low birth weights** were those neonates born with a birth weight of 1500 – 2499g.

Small for gestational age was defined as growth at the 10th percentile or less for weight of all new-borns at that gestational age.

Failure to thrive was defined as weight less than the third to fifth percentile for age on more than one occasion; OR weight measurements that fall two major percentiles using standard growth charts (46).

Appendix 8. Laboratory Procedure for CMV PCR

The dry oral swab was dissolved in Phosphate Buffered Saline (PBS) at least 12 hours before the process of DNA extraction and amplification. 200µl of this solution was then required for the four steps of DNA extraction. The four steps included lysis, binding, washing and elution. The kit used for this process was known as the QIAamp Min Elute Virus Spin Kit.

200µl of the PBS solution containing the dissolved specimen was then pippeted into an eppendorf tube. QIAGEN protease was then added to the solution to breakdown proteins. Lysis buffer; known as buffer AL was also added to the solution. Lysis involved breaking up of the virus cell wall to release DNA. This was done under highly denaturing conditions at high temperatures. After lysis, the solution was then transferred to a spin column. This spin column contained a silica gel membrane. The spin column was put in a centrifuge – Neofuge 13R and centrifuging took place at a speed of 8000 revolutions/minute at room temperature. The centrifuge forced the binding solution through the silica membrane in the spin column. Nucleic acid would bind to the membrane as the solution passed through.

The solution that passed through the membrane was discarded and three wash buffers were added to the spin column; Buffer AW1, Buffer AW2 and ethanol. The column was again put into a centrifuge, forcing the membrane through the wash buffers. This removed any impurities from the membrane. A final dry spin was performed in order to remove any traces of ethanol and other impurities, leaving only the nucleic acid bound to the membrane. Finally, elution buffer – Buffer AVE was then added to the spin column. This was put in a centrifuge again, forcing the membrane through the elution buffer. This washed off the nucleic acid that was bound to the membrane into the bottom of the spin column from where it was collected. This was then stored in an eppendorf tube. The content was pure viral nucleic acid.

The next steps involved nucleic acid amplification. This was done using the CMV Real – TM PCR kit. Nucleic acid amplification had four steps; denaturation of DNA at 95^0C to break the hydrogen bonds holding the 2 strands; therefore forming single stranded DNA (ssDNA). Second step was primer annealing; this required the primers to bind to their complementary sequence in the template. It occurred at temperatures of 45 – 72^0C; third was DNA extension. In this step DNA polymerase extended the primer by adding individual nucleotides onto the primer in a sequential manner, using the target DNA as a template. Temperatures of 72^0C were required for this step. The final step was termination. These steps were repeated severally.

The process began with preparation of the reagents. A total volume of 25µl was required, with 10µl of this being the volume of the DNA sample. The numbers of tubes required for amplification of DNA from

test and control samples were prepared. The reagents to be used were; mix 1, mix 2, polymerase (Taq F) and the buffers. This formed the master mix. Contents in the reagents included: DNA polymerase, primer, deoxynucleoside triphosphates (dNTPs), divalent cations and a buffer to maintain pH. 15µl of the prepared mix was transferred into each tube. Using tips with an aerosol barrier, 10µl of the prepared DNA obtained from the test or control samples was put into the prepared tubes. Control amplification reactions were also carried out by adding 10µl of DNA buffer into the tubes labelled negative control of amplification (NCA), positive control of amplification(C+) and negative control of extraction (NCE).

Programming was then done on the rotor gene™ 3000/6000/Q (Corbett Research Qiagen). FAM, HEX/Cy3/JOE were the filters used to detect viral nucleic acid and the colour shown determined if the nucleic acid was from internal control(IC), the positive control, negative control or the sample. This was according to the manual. Detection as shown on the table was only from cycling 2 which was the extension stage.

Table 8: DNA amplification program for rotor type instruments

STEPS	TEMPERATURE, ^{O}C	TIME	FLUORESCENCE DETECTION	REPEATS
Hold	$95^{0}C$.	15 minutes	-	1
Cycling	95	5s	-	
	60	20s	-	5
	72	15s	-	
Cycling 2	95	5s		
	60	20s	FAM/GREEN JOE/YELLOW	40
	72	15s		

Quality Control Measures

Internal quality control measures were through the use of NCA which was a negative control amplification. It was used as an indicator of perfect nucleic acid extraction , quality of samples and quality of the PCR. It should normally yield a negative result. It allows to check for contamination of the reagents or artefacts that would give a false positive. Positive control of amplification is by use of the C+. The result is already known to be positive, but confirms that there were no mistakes during the procedure.

CMV PCR tests are not among the tests with external quality control at the Kenya AIDS Vaccine Initiative (KAVI) laboratory. However, KAVI laboratory obtained GCLP accreditation, as defined by Good Clinical Laboratory Practice IN 2005. This was the British Association of Research Quality Assurance (BARQA-GCLP).

For quality control, a defined quantity of internal control was introduced into each sample and control at the beginning of sample preparation. A NCE, NCA, C+ were required for every run to verify that the steps were carried out correctly.

Appendix 9. Normal Laboratory Values for Haemoglobin Levels

AGE	HEMOGLOBIN (g/dL)	HEMATOCRIT (%)	MCV (μ^3)	RETICULOCYTES (%)
GESTATIONAL (wk)				
18-20*	11.5 ± 0.8	36 ± 3	134 ± 8.8	N/A
21-22*	12.3 ± 0.9	39 ± 3	130 ± 6.2	N/A
23-25*	12.4 ± 0.8	39 ± 2	126 ± 6.2	N/A
26-27	19.0 ± 2.5	62 ± 8	132 ± 14.4	9.6 ± 3.2
28-29	19.3 ± 1.8	60 ± 7	131 ± 13.5	7.5 ± 2.5
30-31	19.1 ± 2.2	60 ± 8	127 ± 12.7	5.8 ± 2.0
32-33	18.5 ± 2.0	60 ± 8	123 ± 15.7	5.0 ± 1.9
34-35	19.6 ± 2.1	61 ± 7	122 ± 10.0	3.9 ± 1.6
36-37	19.2 ± 1.7	64 ± 7	121 ± 12.5	4.2 ± 1.8
38-40	19.3 ± 2.2	61 ± 7	119 ± 9.4	3.2 ± 1.4
POSTNATAL (DAYS)				
1	19.0 ± 2.2	61 ± 7	119 ± 9.4	3.2 ± 1.4
2	19.0 ± 1.9	60 ± 6	115 ± 7.0	3.2 ± 1.3
3	18.7 ± 3.4	62 ± 9	116 ± 5.3	2.8 ± 1.7
4	18.6 ± 2.1	57 ± 8	114 ± 7.5	1.8 ± 1.1
5	17.6 ± 1.1	57 ± 7	114 ± 8.9	1.2 ± 0.2
6	17.4 ± 2.2	54 ± 7	113 ± 10.0	0.6 ± 0.2
7	17.9 ± 2.5	56 ± 9	118 ± 11.2	0.5 ± 0.4
POSTNATAL (wk)				
1-2	17.3 ± 2.3	54 ± 8	112 ± 19.0	0.5 ± 0.3
2-3	15.6 ± 2.6	46 ± 7	111 ± 8.2	0.8 ± 0.6
3-4	14.2 ± 2.1	43 ± 6	105 ± 7.5	0.6 ± 0.3
4-5	12.7 ± 1.6	36 ± 5	101 ± 8.1	0.9 ± 0.8
5-6	11.9 ± 1.5	36 ± 6	102 ± 10.2	1.0 ± 0.7
6-7	12.0 ± 1.5	36 ± 5	105 ± 12.0	1.2 ± 0.7

6-7	12.0 ± 1.5	36 ± 5	105 ± 12.0	1.2 ± 0.7
7-8	11.1 ± 1.1	33 ± 4	100 ± 13.0	1.5 ± 0.7
8-9	10.7 ± 0.9	31 ± 3	93 ± 12.0	1.8 ± 1.0
9-10	11.2 ± 0.9	32 ± 3	91 ± 9.3	1.2 ± 0.6
10-11	11.4 ± 0.9	34 ± 2	91 ± 7.7	1.2 ± 0.7
11-12	11.3 ± 0.9	33 ± 3	88 ± 7.9	0.7 ± 0.3
12-14	11.9	37	86.8	0.9

Appendix 10. Time Frame

The following is a proposed time-frame of the study process:

Number	Activity	Estimated Time
1	Proposal Development and Presentation	November to March 2016
2	Submission of proposal for ethical approval	March - April 2016
3	Piloting of CRF	May 2016
4	Data Collection and data entry	June to September 2016
5	Data cleaning and analysis	October to November 2016
6	Thesis submission	August 2017
7	Thesis defense	October 2017

Appendix 11. Study Budget

Category	Remarks	Units	Unit Cost (KShs)	Total (KShs)
Proposal Development	Printing drafts	1000 pages	5	5,000
	Proposal Copies	8 copies	500	4,000
Data Collection	Stationery Packs (Pens, Paper and Study Definitions)	10	100	1000
	Training research assistants	1 day	1000	1, 000
	Research assistants (2)	12 weeks	1000 X 2	24, 000
Lab technique – and oral swabs	- Real time PCR - QIAamp MinElute Virus spin - Bench fee	2 4 1	60,000 33,048 20,000	550,000
Thesis Write Up	Computer Services			5,000
	Printing drafts	1000 pages	5	5,000
	Printing Thesis	10 copies	500	5,000
Contingency funds				20,000
Total				**620,000**