PREVALENCE OF HYPOCALCAEMIA IN TERM NEONATES AND THE CLINICAL CHARACTERISTICS OF THE AFFECTED NEONATES ADMITTED TO THE NEONATAL INTENSIVE CARE UNIT AT THE UNIVERSITY TEACHING HOSPITAL

BY

KHOZYA DAVIE ZYAMBO

(BScHB, MBChB)

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DEDICATION

To my wife, Tiwonge M. Zyambo, your unwavering support is what I was riding on through this spirited journey to this happy end. I am deeply thankful. To my three children, Tikhozye, Wankhongono and Zitube-Wane, I picked up a lot of paediatric lessons from watching you grow. I love you all.

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AUTHOR OF DECLARATION

I declare that this dissertation is my own work. It is being submitted for the Master's degree in Paediatrics and Child Health at the University of Zambia, Lusaka. It has not been submitted before for any degree or examination at this or any other University.

Candidate

Dr. Khozya Davie Zyambo

(BScHB, MBChB)

Principal supervisor

Dr. Silvester Sinyangwe

(MBChB, MMED, MPH)

Co-supervisor

Dr. Chishala Chabala

(BScHB, MBChB, MMed, MSc. Clinical Trials)

CERTIFICATE OF APROVAL

The University of Zambia approves this dissertation by **Khozya Davie Zyambo** as partial fulfillment towards the requirement for the award of the degree of Master of Medicine of Paediatrics and Child Health.

Head of Department

_____

Name Signed Date

Examiner- 01

_____

Name Signed Date

Examiner- 02

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Name Signed Date

Examiner- 03

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Name Signed Date

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ABSTRACT

Convulsions with concurrent hypocalcaemia are a common complication seen in very ill neonates at The University Teaching Hospital (UTH) Neonatal Intensive Care Unit (NICU) which admits close to 4000 neonates per year with various neonatal conditions. Convulsions in hypocalcaemic neonates have been found to be unamenable to anti-epileptic therapy thus leading to persistent convulsions which seem only to be controlled once calcium gluconate is administered to correct the hypocalcaemia. No studies in Zambia have been done to determine the burden of neonatal hypocalcaemia. Meanwhile, studies around the globe have shown prevalence levels of neonatal hypocalcaemia ranging from 17% to 39%. This study endeavored to determine the prevalence of hypocalcaemia in term neonates, the clinical characteristics of the affected neonates and to establish the proportion of neonates with seizures who also have hypocalcaemia.

This was a cross sectional study with a sample size of 174 neonates (calculated using Epi info version 3.5.1 at power of 80%) who were enrolled to the study by simple random sampling methods out of 240 screened neonates. Data were analyzed using the statistical software package SPSS version 2. The relationship between study variables and outcome variable of interest (hypocalcaemia) was examined using logistic regression model which was considered at $P < 0.20$ significance.

The prevalence of neonatal hypocalcaemia was found to be 26.4% (46/174) and 50% of these neonates presented with neurological disorders such as convulsions, hypertonia and hypotonia. Of the neonates who presented with convulsions in this study, 26.8% (19/71) were also found to have hypocalcaemia. However, there was no statistically significant association between convulsions and hypocalcaemia.

The prevalence of hypocalcemia in term neonates admitted to NICU at UTH is high with half of the hypocalcaemic neonates manifesting neurological signs. Early identification and in turn treatment of hypocalcaemia in the neonates is paramount in achieving prompt interventional care of severely ill term neonates at increased risk of electrolyte imbalance. It is highly recommended to add to the essential list of investigations the measurement of serum calcium in the NICU.

Key words- hypocalcaemia, convulsions, term neonates,

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ABBREVIATIONS

NICU	Neonatal Intensive Care Unit
UTH	University Teaching Hospital
UNZA	University of Zambia
LNMP	Last Normal Menstrual Period
ENH	Early Neonatal Hypocalcaemia
LNH	Late Neonatal Hypocalcaemia
PICU	Paediatric Intensive Care Unit
QTc	Corrected QT interval on the electrocardiographic tracing
WHO	World Health Organization
IDM	Infant of a Diabetic Mother

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Hypocalcaemia in the first 3 days is known as Early Neonatal Hypocalcaemia (ENH) whereas hypocalcaemia occurring in the second week of life is referred to as Late Neonatal Hypocalcaemia (LNH) commonly caused by ingestion of cow's milk which has a high content of phosphates **(Wandrup *et al*, 1988).**

In utero, active calcium transfer from maternal circulation to the fetus is facilitated by parathyroid hormone (PTH) via a transplacental calcium pump mainly during the third trimester of pregnancy. As a result total serum calcium concentration in the fetus is higher than that of the mother with total serum calcium levels in umbilical cord blood reaching 2.5–2.75 Mmol/L **(Wandrup *et al*, 1989).** However, Placental transfer stops abruptly after birth. Within the first few hours of life, the newborns undergo a physiological drop in serum calcium levels by 24-72 hours of age. Then the calcium level increases to normal values by the tenth day of life **(Loughead *et al*, 1988; Husain *et al*, 1993)**. This homeostatic state is achieved by the tight regulation of the following factors; PTH secretion, dietary calcium intake, renal calcium reabsorption, skeletal calcium stores, and vitamin D levels.

In high risk neonates; low birth weight, birth asphyxia, and infants of a diabetic mothers **(Tsang *et al*, 1971, 1973, 1974; Jain *et al*, 2008)**, early neonatal hypocalcaemia is usually worsened necessitating treatment with calcium supplementation for at least the first 72 hours **(Jain *et al*, 2008)**.

Normal levels of serum calcium vary with gestation age of the newborn. Hypocalcaemia is defined as ionized calcium <1.22 Mmol/L or total serum calcium <2.21 Mmol/L **(Jain *et al*, 2008)** in term neonates. Preterm infants generally are not considered to have hypocalcaemia until serum total calcium values fall below 1.8 Mmol/L, **(Zhou *et al*, 2009).**

Ionized calcium is essential for a number of biochemical processes in the body. These include, blood coagulation as a co-factor, neuromuscular excitability, cell membrane integrity and function, and cellular enzymatic and secretory activity (**Wandrup *et al*, 1988; Wandrup *et al*, 1989)**. Hypocalcaemia in neonates therefore, is potentially a life-threatening condition and may

present with signs of neuromuscular irritability; myoclonic jerks, twitching of muscles and extremities, seizures and other none specific signs like apnoea, cyanosis, high pitched cry and/or cardiac rhythm disturbances (prolonged QTc) **(Wandrup *et al*, 1988)**.

Its prevalence has been observed to vary with gestational age, perinatal factors, neonatal and maternal comorbidities. In one European study, it was found to be as high as 39% **(Roberton *et al*, 1975).**

The University Teaching Hospital (UTH) Neonatal Intensive Care Unit, (NICU) Zambia's largest referral hospital based in Lusaka - the capital city, admits close to 4000 neonates per year with various neonatal conditions (NICU 2012 and 2013 annual reports). One of the important neonatal conditions observed in NICU is neonatal hypocalcaemia (NICU 2012 and 2013 quarterly mortality audits).

According to the NICU protocol at UTH, serum calcium measurement is one of the important baseline electrolyte tests that should be ordered in very ill neonates, however the test is not routinely run at the hospital laboratory due to unavailability of reagents. As a result, clinicians encounter difficulties in promptly identifying and managing hypocalcaemia in the neonates. Anecdotal reports in NICU at UTH have shown that severely ill neonates (asphyxia, meningitis, acute respiratory distress syndrome) presenting with seizures and hypocalcaemia concurrently, respond poorly to anti-epileptic drug therapy. Despite use of recommended doses of anti-epileptic drugs, convulsions persist until several doses of calcium gluconate are given empirically.

Clinicians in NICU are mostly prompted to investigate for serum calcium when neonates development clinical features of hypocalcaemia and/or when fits in the neonates do not response to anti-epileptic drugs. Unfortunately, serum calcium studies usually have to be done at a private laboratory and only a few patients have the ability to pay the fees for these services. As a result, hypocalcaemic levels remain undetermined in most of the neonates with fits for a while before presumptive treatment with calcium gluconate is instituted to control the fits. The delay in identifying and treating hypocalcaemia in the neonates, thus contributes greatly to the neonates' prolonged stay in hospital and increasing their chances of acquiring nosocomial infections.

This study seeks to establish the extent of the problem of hypocalcaemia in term neonates and document the neonatal conditions that are commonly associated with it.

1.2 STATEMENT OF THE PROBLEM

Measurement of serum calcium levels for a resource constrained hospital such as UTH, with a stringent financial budget, is not a routine practice despite it being an important test as recommended in the NICU protocol (the UTH laboratory seldom have reagents to run the test). WHO does recognize that measurement of serum ionized calcium in hospital settings is less easily available than measurement of blood sugar **(WHO, 2011).** This inability to run routine serum calcium levels has led to delays in establishing hypocalcaemia in affected neonates and subsequently its prompt management. It can be argued that one of the reasons that can explain the laboratory's inability to run serum calcium studies as routine tests is the lack of evidence (University of Zambia-UNZA library and internet search-PubMed and Cochrane library, showed no records of studies done at UTH or around the region on this subject) to demonstrate the burden of morbidity associated with hypocalcaemia in neonates to justify the costs involved.

Investigations for suspected hypocalcaemia are ordered late, many times prompted by clinical signs and non-response of convulsions to anticonvulsants. Babies with hypocalcaemia are therefore identified late and appropriate treatment is often delayed, as a result newborn babies are subjected to prolonged stay in hospital with increased risk of acquiring nosocomial infections.

1.3 STUDY JUSTIFICATION

There had been no studies done to demonstrate the prevalence of neonatal hypocalcaemia at UTH.

It was envisaged that the findings in this study will, therefore, provide an evidence based local resource of information as a basis for reviewing and designing NICU treatment protocols in terms of managing electrolyte imbalances and also provide the needed evidence to support/justify routine measurement of serum calcium in all high risk neonates admitted to NICU despite the associated high costs of the test.

This will ensure early identification of hypocalcaemia in at-risk neonates and necessitating early initiation of appropriate interventional measures to correct the electrolyte disorder. This will then improve morbidity outcomes, reduce the duration of stay in hospital and

consequently, in the long term, reduce the risk of neurological sequelae that may result from prolonged uncontrolled fits.

Moreover, most of the available literature cited in this study surrounding this topic is old with paucity of information on the current trends, (WHO, 2011). It was therefore, hoped that the findings in this study will add to the improvement of care and promote evidenced based approach to the care of neonates in NICU at UTH.

1.4 RESEARCH QUESTION

What is the prevalence of hypocalcaemia in term neonates that are admitted to UTH NICU and what are the clinical characteristics of the neonates affected with hypocalcaemia?

1.5 OBJECTIVES

1.5.1 General Objective

To determine the prevalence of hypocalcaemia in term neonates and the clinical characteristics of the affected neonates.

1.5.2 Specific Objectives

i. To determine the prevalence of hypocalcaemia within the first 5 days of life among term neonates admitted to NICU.
ii. To determine the clinical characteristics of the neonates with hypocalcaemia.
iii. To determine the proportion of neonates with seizures who also have hypocalcaemia.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 EPIDEMIOLOGY

The prevalence of hypocalcaemia in neonates has varied across the globe as shown in several studies done in different regions. **Roberton *et al* (1975),** in a study found that 51 infants out of 131 (39%) had early neonatal hypocalcaemia while another study by **Tsang *et al*, (1970)**, during an 8-month period, found that 37 out of 124 low birth weight (LBW) infants (29.8%) admitted to nursery developed hypocalcaemia in the first 3 days of life. Our study however, investigated the prevalence of hypocalcaemia among term neonates in the first week of life.

The prevalence of neonatal hypocalcaemia with seizures has been reported in a number of studies published in the 1970s **(Keen *et al*, 1973; Langevin *et al*, 1974)** varying between 2.3% to 9%. It is postulated that better nutritional management may have led to a decline in the incidence of hypocalcaemia in more recent times. However, available data is insufficient to prove this assumption **(WHO, 2011)**. **Rose *et al* (1970)**, found the prevalence of hypocalcaemia in term neonates weighing more than 2.5Kg with seizures to be 20.4%.

Furthermore, the impact of hypocalcaemia in critically ill patients can be seen from the study by **Broner *et al* (1990)**, who examined serum ionized calcium and magnesium in paediatric patients consecutively admitted to Paediatric Intensive Care Unit (PICU) and found 17% had hypocalcaemia which, together with hypermagnesaemia, was associated with poor outcome in terms of both morbidity and mortality. This merely demonstrates the impact calcium and magnesium imbalances have on morbidity and mortality among patients in intensive care units.

A more recent study by **Thomas *et al*, (2012)** revealed a prevalence of 0.02% of late onset hypocalcaemia in term neonates <31days old seemingly to suggest great strides have been taken to reduce the prevalence of hypocalcaemia in neonates.

2.2 ETIOLOGY

Hypocalcaemia in neonates may result from a number of clinical problems a newborn is likely to encounter in the first 3 days of life. Some etiological considerations include; reduced calcium supply, increased endogenous phosphate load, hypomagnesaemia, alkali therapy, functional

hypothyroidism, defective vitamin D metabolism and possibly calcitonin excess mainly leading to early neonatal hypocalcaemia, **(Tsang et al, 1977).**

Some studies have observed subnormal maternal serum calcium levels during pregnancy **(Khattab *et al*, 1970)** resulting in an increased risk of their newborns developing hypocalcaemia. However, follow up studies have demonstrated no significant differences in maternal serum ionized calcium levels in postpartum mothers whose newborns had prolonged convulsions compared to the mothers whose newborns did not have convulsions **(Khattab *et al*, 1971)**. Vitamin D deficiency during pregnancy and lactation can lead to hypocalcaemia and rickets in neonates and infants, **(Camadoo *et al*, 2007; Kovacs, 2008)**. Neonates born to vitamin D deficient mothers are at a significantly higher risk to develop hypocalcaemic seizures **(Mehrotra *et al*, 2010).**

2.3 TREATMENT/PREVENTION

In correcting hypocalcaemia with intravenous calcium gluconate, it has been documented that significant harm such as asystole or skin necrosis may result. The benefit versus harm ratio for empirical treatment of hypocalcaemia before laboratory tests cannot be assessed **(WHO, 2011)**. This may have led to the on-going debate on whether to administer prophylactic calcium gluconate to all at risk neonates or to only give treatment to neonates with established hypocalcaemia.

One study has proposed that neonatal hypocalcaemia can be abated if high-risk infants are prophylactically given calcium supplementation during the high risk period (first 3 days of life), especially the infants who are receiving intravenous fluids with low oral calcium intake and requiring $NaHCO_3$ for correction of acidosis **(Tsang *et al*, 1970)**. While **Roberton *et al*, (1975)** later on suggests that all sick low birth weight infants should have daily serum calcium estimations carried out and calcium supplements should only be considered if symptoms of hypocalcaemia are present.

2.4 COMPLICATIONS

In the presence of biochemical disturbances, it is difficult to achieve proper control of seizures and thereby increasing the risk of further brain damage. Early recognition and treatment of biochemical disturbances are essential for optimal management and satisfactory long-term outcome, **(Kumar et al 1994).**

A more recent study done demonstrated that delayed intervention in treating hypocalcaemia may lead to neurological sequelae especially in neonates with concurrent seizures and hypocalcaemia, **(Evelyn *et al,* June 2013)**. This study showed that neonatal seizures in 22q.11.2 deletion syndrome, mediated by neonatal hypocalcaemia, increased the risk for severe intellectual deficits in affected children and observed that neonatal hypocalcaemia often remained unrecognized until the post seizure period, when damage to neurons may already have occurred hence supporting and necessitating early identification and treatment of neonatal hypocalcaemia.

CHAPTER THREE

3.0 METHODOLOGY

3.1 STUDY TYPE

This was a cross sectional study.

3.2 STUDY SITE

The study was conducted at the Neonatal Intensive Care Unit at University Teaching Hospital in Lusaka, Zambia. The hospital is known to admit an average of 4000 newborns per year, 2900 of which are term neonates (2012 and 2013 NICU medical records). The unit receives patients from the UTH labour ward and various health centres in Lusaka. It has a bed capacity of 90 with 28 incubators. It is composed of the following staff; 1 consultant paediatrician, 2 senior registrars, 3-4 registrars, 1-2 resident interns, 16 nursing staff, 3 pharmacists, 1-2 pharmacy interns and nutritionists.

3.3 STUDY POPULATION

The study included all term neonates less than 5 days old admitted to NICU at UTH for various neonatal conditions requiring intensive care. These included referrals from the UTH labour ward and from various health centres in Lusaka urban.

3.4 ELIGIBILITY CRITERIA

3.4.1 Inclusion criteria

- Term neonates who were 37 weeks or more by gestation age. (The Ballard scores were used in cases where the LNMP dates were uncertain).
- Neonates who were less than 5 days old.
- Parental signed consent.

3.4.2 Exclusion criteria

- Prematurity i.e. 36 weeks gestation age or less.
- Congenital anomalies.
- Failure to collect blood from the peripheral veins.
- Declined consent.

3.5 SAMPLING

Simple random sampling methods was employed to recruit study participants as detailed in section 3.7 below.

3.6 SAMPLE SIZE

Sample size was calculated using Epi info version 3.5.1 at power of 80% with an assumed study population of 2900 term neonates admitted to NICU every year and the assumed neonatal hypocalcaemia prevalence of 40%. The worst expected sample population prevalence of 32.5%. The sample size calculated was **164.** However, the actual number of research participants included in this study came to **174** neonates.

3.7 SAMPLING PROCEDURE

Once the eligible patients were identified through a screening process, they were assigned numbers from 1 to 6 (depending on the number of identified eligible participants). To select a participant from the eligible group, a dice was rolled to give a random number. Then the patient with the number corresponding to the number on the rolled dice would be approached to obtain informed consent and included in the study once they consented after going through the information sheet with them by appending their signature or thumb print on the consent form. Those who declined to consent were dropped off the study.

3.8 STUDY PROCEDURE

The information sheet was either given or read to potential study participants who would then either consent or decline. The process of consenting was undertaken in the preferred language of the potential participant. For those who were not able to read, an impartial witness was present through the consenting process. Once the consent was obtained, the study questionnaire was administered and a physical examination conducted. A maximum of 2mls of blood sample was collected in a lithium heparin container from the peripheral veins. The samples where then coded, packed in a courier cooler box and sent to the UTH laboratory within 2 hours of having collected the blood. The following tests were requested; corrected serum calcium, sodium, phosphate and magnesium. Other tests which were performed in the study included; haematocrit and random blood sugar (bed side tests that involved a needle prick at the heel of the foot). The results once obtained were availed to the attending clinician for appropriate action.

3.9 OUT COMES

- Hypocalcaemia i.e. corrected serum calcium level <2.10 Mmol/L
- Documented convulsions in neonates with hypocalcaemia

3.10 INDEPENDENT VARIABLES

- Sex
- Gestation age
- HIV status
- Asphyxia
- Sepsis
- Infant of a Diabetic Mother (IDM)
- Fever
- Hypoglycaemia
- Magnesium
- Sodium

3.11 DATA MANAGEMENT

A standardized data entry questionnaire was used for each research participant for collection of data and the data was entered and aggregated on an Epi Info database.

3.12 DATA ANALYSIS

Data were analyzed using the statistical software package SPSS version 21. All statistical tests were at 5% significance level. The Independent Samples T-test was used to compare mean values between groups, the Pearson's chi-squared and Fisher's exact test was used for comparison of proportions between groups. The relationship between study variables and outcome variable of interest (hypocalcaemia) was examined using logistic regression. Selection for logistic regression model was considered at level $P < 0.20$ or known clinical significance. Backward selection method was used to obtain the final logistic regression model. The backward selection method removes terms one at a time beginning with the largest p-value and continuing until all remaining effects are significant at a specified level or removing more terms results in poorer fit.

3.13 ETHICS

Ethical clearance was obtained from ERES CONVERGE IRB. The permission to conduct the study at UTH was granted by Department of Paediatrics and Child Health and the UTH management.

The purpose and procedures of the study was clearly explained in the preferred language of the research participants (parent/guardian) and a signed written informed consent was obtained. It was emphasized during the consenting process that participation in the study was voluntary and that participants were at liberty to withdraw from the study at any point without being denied continued medical care.

The risks and benefits of the study were fully and clearly explained to the research participants as described in the consent form. Highlighted possible risks included, delayed hemostasis from the venipuncture site especially in neonates at risk of bleeding tendencies such as severe sepsis as well as pain due to needle prick. The anticipated benefits included free serum calcium tests, early detection of electrolyte abnormality and quick referral for appropriate care.

The participant's results and other clinical information were kept strictly confidential and were only disclosed to the attending doctor for appropriate action. All data entry forms were coded and no names were used. The data entry sheets were kept in a locked secure cabinet and all electronic entries were password protected.

Participants who needed urgent treatment or further follow up were referred appropriately.

CHAPTER FOUR

4.0 RESULTS

A total of 240 patients were screened over a period of six months (December 2014 to May 2015). Of these, 200 patients met the eligibility criteria but only 179 patients where successfully recruited into the study while the rest of the 21 were left out for various reasons such as declined consent and missing results. The study was enrolling 1 to 4 patients per day, between 08:00 hours to 16:00 hours from Monday to Friday.

There were a total of 179 term neonates enrolled to this study and only 174 participants with serum calcium results were finally analyzed (figure 1).

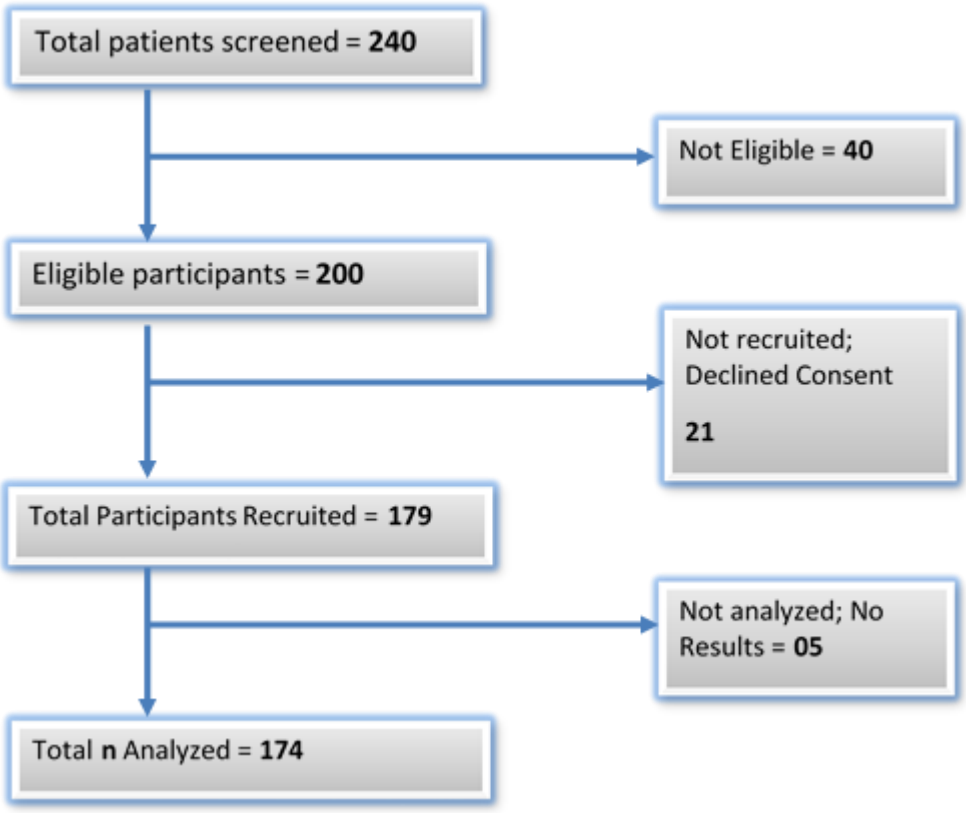

Figure 1. Research Participants Recruitment Flow Chart

4.1 CHARACTERISTICS OF THE STUDY POPULATION

The mean age of the study population was 1.3 days (SD = 0.60) while the male to female ratio was 1.4:1; 101/174 (58%) male neonates and 73/174 (42%) female neonates. Sixty nine (39.7%) neonates had birth weights ranging between 2.5 to 3.0 Kg while 93 (53.4%) were

between 3.1 to 3.9 Kg and 12 (6.9%) were macrosomic, with birthweight over 3.9 Kg. The mean gestation age was 38.8 ± 1.24 weeks by dates.

The most common reason for referral to UTH NICU for the neonates was asphyxia 62/174 (35.6%) followed by fever 25/174 (14.4%) while the most common clinical diagnosis made in the admitted neonates was asphyxia 59 (33.9%) and sepsis 58 (33.3%). Figure 2 shows a bar chart for neonatal diagnosis and Table 1 shows the reasons for referral frequency distribution.

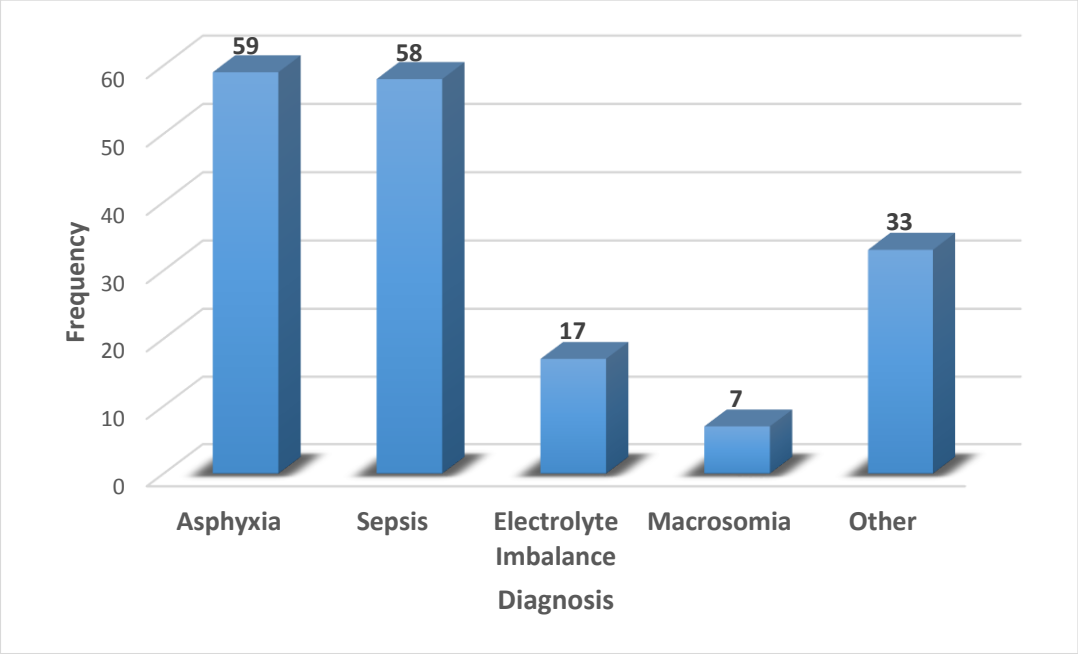

Figure 2. Neonatal Diagnosis Bar Graph

Table 1. Reasons for Referral Frequency Distribution

REASON FOR REFERRAL	FREQUENCY	PERCENT %
Asphyxia	62	35.6
Fever	25	14.4
Convulsions	18	10.3
Sepsis	16	9.2
Fast breathing	12	6.9
Big baby	9	5.2
Hypothermia	6	3.4
Birth trauma	5	2.9
Aspiration	4	2.3
Infant of a diabetic mother	3	1.7
Post mature	3	1.7
Other	11	6.3
TOTAL	**174**	**100**

The following clinical features were observed in the sampled population; 12 (6.9%) neonates had jaundice, 22 (12.6%) had cyanosis and 16 (9.2%) had abnormal skin findings. There were 98 (56.3%) neonates with normal heart rate, while 71 (40.8%) had tachycardia, and 5 (2.9%) neonates with bradycardia. There were 85 (48.9%) neonates with normal respiratory rate, 84 (48.3%) with tachypnoea, and 5 (2.9%) with brachypnoea. There were 43 (24.7%) neonates with normal body temperature whereas 32 (18.4%) had hyperthermia and 99 (56.9%) had hypothermia. The general appearance for majority of the children was ill 130 (74.7%) and only 44 (25.3%) appeared well. There were only 16 (9.2%) neonates with pallor and the rest (90.8%) had no pallor present. About one-thirds of the neonates had Apgar score ≥ 7 at birth, and 87 (50%) had Apgar score ≥ 7 at 1 minute, and 97 (55.7%) had Apgar score ≥ 7 at 5 minutes.

Systemic clinical examination of the neonates showed that the majority, 172 (98.9%) had normal cardiovascular findings. It was also noted that majority of the neonates had normal respiratory findings, 169 (97.1%) and normal abdominal findings, 172 (99.9%). Only 24 (13.8%) neonates were found to be HIV exposed and the rest (86.2%) were not.

4.2 MATERNAL CHARACTERISTICS

Maternal age had a mean of 24.9 years (SD = 6.60) as shown in Table 2. Maternal parity was right skewed with a median of 1 (min = 1, max = 8). There were 24 (13.8%) mothers with a positive HIV status and 150 (86.2%) had a HIV negative status. There were only 5 (2.9%) mothers with diabetes mellitus while a majority 169 (97.1%) did not have diabetes mellitus.

Table 2. Maternal Age Summary Statistics

N	MEAN	STD. DEVIATION	MEDIAN	MINIMUM	MAXIMUM
174	24.92	6.60	24	13	44

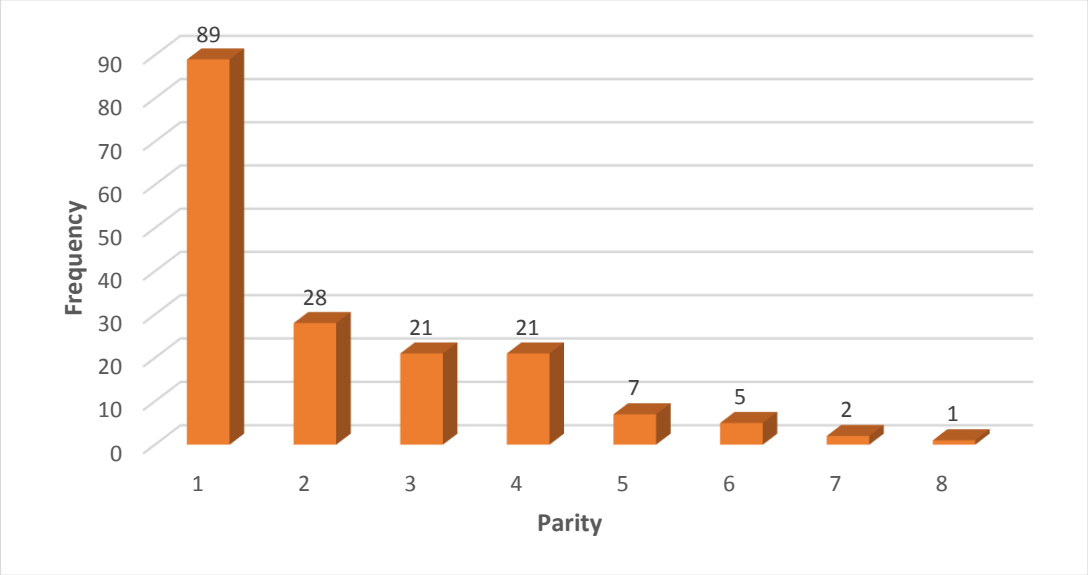

Figure 3. Maternal Parity Frequency Distribution

4.3 LABORATORY TEST RESULTS

Of the 179 neonates enrolled in to the study, 174 had corrected serum calcium test results available for analysis. Figure 4 shows 98 (56.3%) neonates had normal calcium levels, 46 (26.4%) had hypocalcaemia and the rest had hypercalcaemia. Table 3 shows summary statistics of the rest of laboratory tests done on the neonates in this study.

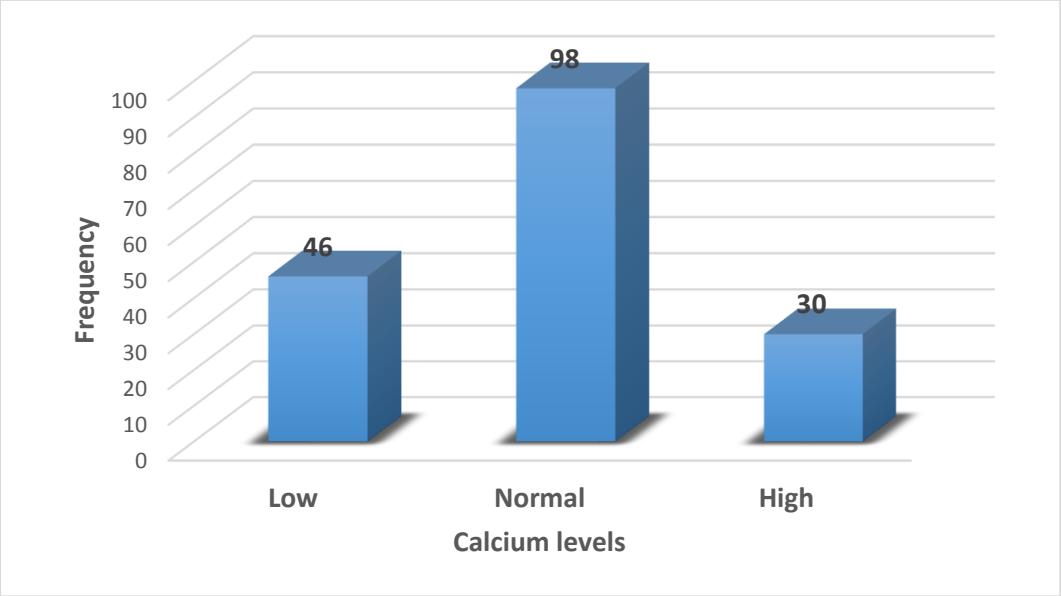

Figure 4. Bar Graph of Neonate Calcium Leve

Table 3. Summary Statistics of Laboratory Test Results

Test	N	Standard Deviation	Median
Random blood sugar	174	3.40	4.70
Calcium	174	0.39	2.18
Magnesium	171	0.39	0.95
Phosphate	148	1.23	2.31
Sodium	163	9.49	139
Haematocrit	174	6.39	49
Platelets	144	93.09	245
Neutrophils	103	5.49	11.30 x 10^3
Leucocytes	144	7.25	17.05 10^3

Note however, that there were only 171 serum magnesium, 163 serum sodium and 148 serum phosphate results available for analysis. Figure 5 shows that 93 (54%) neonates had normal magnesium levels, 64 (37%) had hypermagnesaemia and 14 (9%) neonates had hypomagnesaemia.

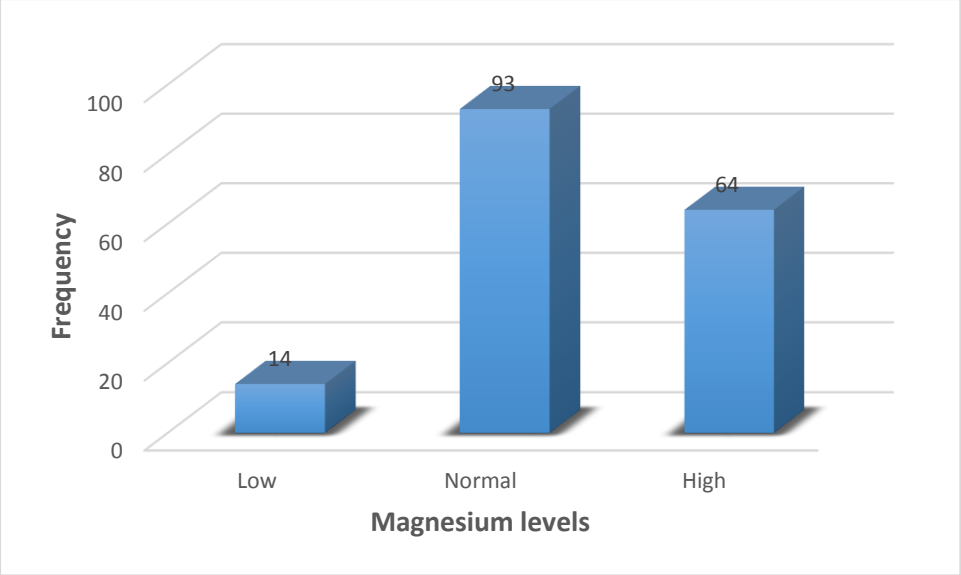

Figure 5. Bar Graph of Neonate Magnesium Level

4.4 BIVARIATE ANALYSIS FOR CATEGORICAL AND CONTINUOUS VARIABLES

There was no association between hypocalcaemia and Apgar score at birth, at 1 minute, and at 5 minutes, P = 0.47, 0.78, and 0.62, respectively. At 5% significance level clinical diagnosis was associated with hypocalcaemia, P < 0.01 and magnesium was marginally associated with hypocalcaemia, P = 0.06. Table 4 and Table 5 show the bivariate analysis for the study categorical variables and continuous variables, respectively.

Table 4. Categorical Variables Bivariate Analysis

Variables	Hypocalcaemia		No Hypocalcaemia		P-Value
	n	**%**	**n**	**%**	
Sex					
Male	26	56.5%	75	58.6%	0.81
Female	20	43.5%	53	41.4%	
HIV status					
Negative	41	89.1%	109	85.2%	0.50
Positive	5	10.9%	19	14.8%	
Maternal parity					
One	21	45.7%	68	53.1%	0.38
Two or more	25	54.3%	60	46.9%	
Neurology					
Normal	23	50.0%	62	48.4%	0.86
Not normal	23	50.0%	66	51.6%	
Diagnosis					
Electrolyte imbalance	11	23.9%	6	4.7%	**< 0.01**
Asphyxia	13	28.3%	46	35.9%	
Sepsis	16	34.8%	42	32.8%	
Macrosomia	0	0.0%	7	5.5%	
Other	6	13.0%	27	21.1%	

Table 5. Continuous Variables Bivariate Analysis

Variables	Hypocalcaemia (n = 46)	No hypocalcaemia (n = 128)	P-value
	mean (SD)	**mean (SD)**	
Age	1.4 (0.75)	1.3 (0.54)	0.22
Birth weight	1.7 (0.60)	1.7 (0.61)	0.97
Gestation age	38.54 (1.07)	38.8 (1.30)	**0.19**
Haematocrit	48.6 (7.69)	48.7 (5.89)	0.88
Platelets	236.2 (125.50)	249.2 (80.47)	0.57
Sodium	135.8 (13.52)	139.0 (7.38)	**0.15**
Phosphate	2.5 (1.06)	2.7 (1.30)	0.31
Magnesium	1.19 (0.51)	1.03 (0.34)	**0.06**
Random blood sugar	5.8 (3.54)	5.8 (3.37)	0.95

4.5 NEUROLOGICAL FINDINGS IN THE STUDY POPULATION

There were 89/174 (51.1%) neonates with neurology disorders. Figure 6 shows the frequency distribution of the neurological disorders found in the neonates. The most common neurological disorder seen in the study were convulsions, 71/174 (40.8%), followed by hypertonia 14 (8.0%).

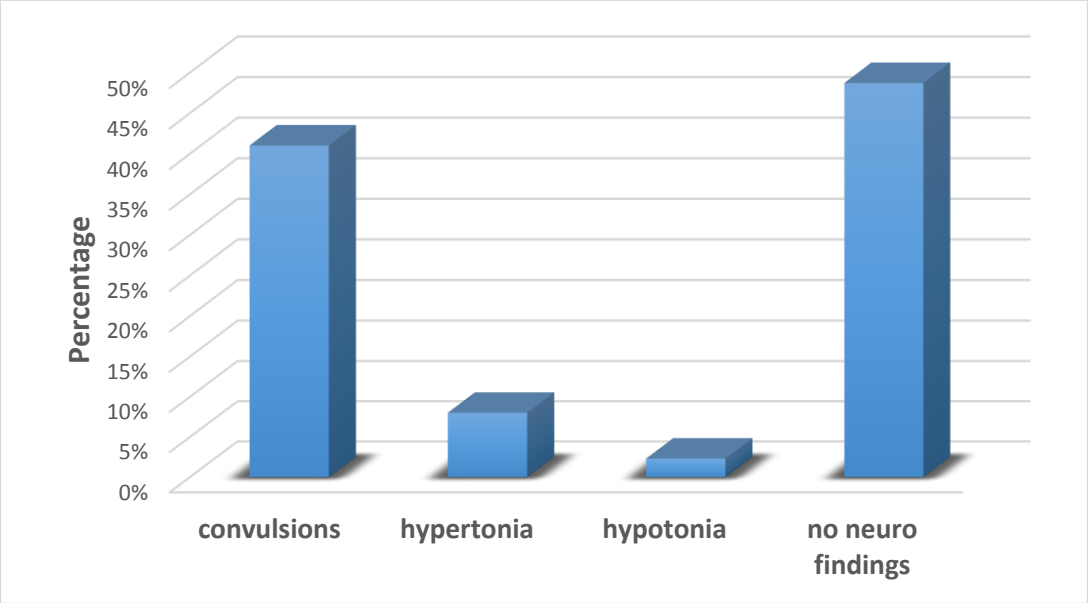

Figure 6. Neurology Disorder Frequency Distribution

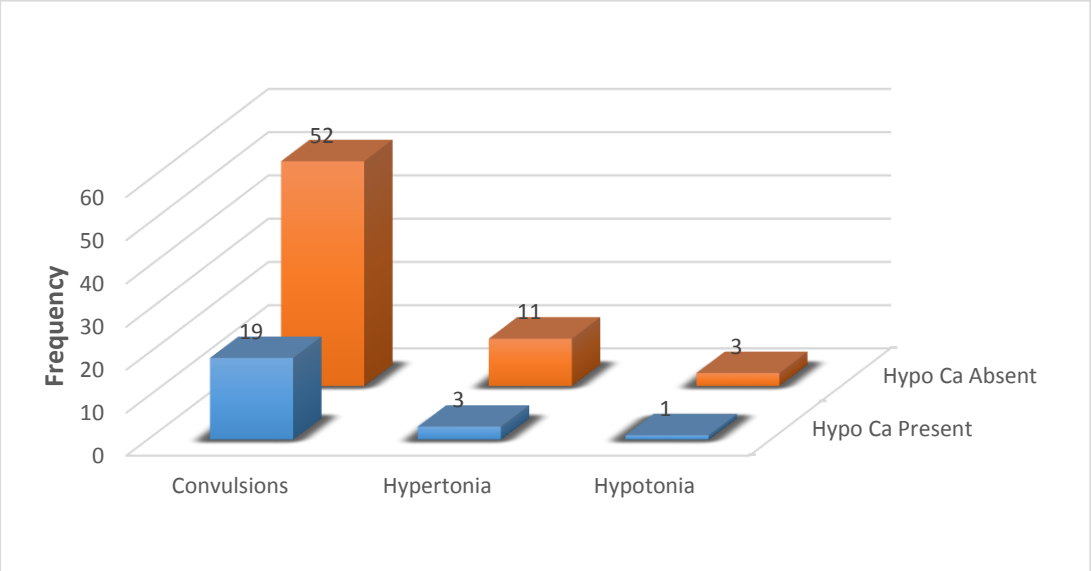

Figure 7. Neurological Disorder in Neonates vs Hypocalcaemia Cross-tabulation

It was observed that 19/71 (21.3%) of the neonates with convulsions also had hypocalcemia (Figure 7). However, there was no significant association between overall neurology status and hypocalcaemia (P = 0.86) and neither was there significant association between neurological disorder and hypocalcaemia (P = 0.60).

Table 6. Frequency Distribution of Neurological Disorders

Neurology disorder (n = 89)		Hypocalcaemia		Total	P value
		Yes	No		
Convulsions	N	19	52	71	0.99
	%	26.8%	73.2%	100.0%	
Hypotonia	N	1	3	4	-
	%	25.0%	75.0%	100.0%	
Hypertonia	N	3	11	14	-
	%	21.4%	78.6%	100.0%	
Total	N	23	66	89	
	%	25.8%	74.2%	100.0%	

Further analysis of the results revealed that 23/46 (50%) of the neonates with hypocalcaemia had neurological abnormalities as noted in Figure 8. There was however no statistically significant association between hypocalcaemia and convulsions (P = 0.99).

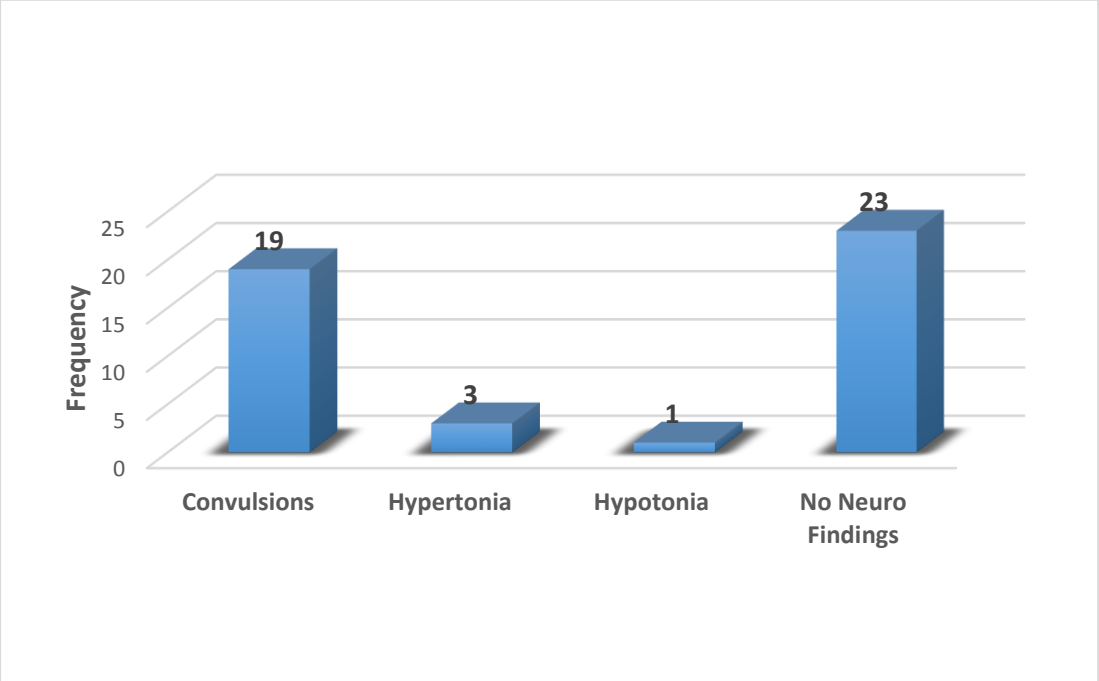

Figure 8. Neurological Findings of Neonates with Hypocalcaemia

4.6 MULTIVARIATE LOGISTIC REGRESSION ANALYSIS

Child age, gestation age, sodium, magnesium, and clinical diagnosis were entered into a multivariate logistic regression model and the backward selection method executed. The resulting model predicting hypocalcaemia is shown in Table 7.

Adjusting for gestation age and clinical diagnosis the odds for hypocalcaemia increased 3 times for every 1 unit increase in magnesium level (OR = 3.11, CI = 1.24 – 7.82, P = 0.02). Adjusting for magnesium and clinical diagnosis the odds for hypocalcaemia reduced by 27% for every 1 week increase in gestation age (OR = 0.73, CI = 0.52 – 1.03, P = 0.07). Adjusting for magnesium levels and gestation age, neonates with electrolyte imbalance had 11 times increased odds for hypocalcaemia compared to neonates with other clinical diagnosis (OR = 11.33, CI = 2.69 – 47.77, P < 0.01). Although not statistically significant, neonates with asphyxia had 24% increased odds for hypocalcaemia (OR = 1.24, CI = 0.43 – 3.74, P = 0.73) compared to neonates with other clinical diagnosis. Neonates with sepsis had 2 times increased odds for hypocalcaemia (OR = 2.06, CI = 0.65 – 6.56, P = 0.22) compared to neonates with other clinical diagnosis, however this was not statistically significant.

Table 7. Multivariate Logistic Regression Predicting Hypocalcaemia

Variable	Crude odds ratio (95% ci)	Adjusted odds ratio (95% ci)	P-value
Magnesium	2.56 (1.12 - 5.85)	3.11 (1.24 - 7.82)	**0.02**
Gestation age	0.83 (0.62 - 1.10)	0.73 (0.52 - 1.03)	0.07
Diagnosis			
Other	1	1	
Electrolyte imbalance	8.25 (2.18 - 31.23)	11.33 (2.69 - 47.77)	**< 0.01**
Asphyxia	1.27 (0.43 - 3.74)	1.24 (0.37 - 4.11)	0.73
Sepsis	1.71 (0.60 - 4.93)	2.06 (0.65 - 6.56)	0.22

CHAPTER FIVE

5.0 DISCUSSION

The prevalence of Early Neonatal Hypocalcaemia (ENH) in term neonates admitted to UTH NICU was found to be 26.4%. Meaning one in every four term neonates admitted to the UTH NICU is likely to have hypocalcemia. Of the neonates with hypocalcaemia, 50% presented with neurological disorders (convulsions, hypertonia and hypotonia). Forty one percent of the neonates in this study presented with convulsions and 26.8% of these neonates had hypocalcaemia. Further statistical tests revealed no statistically significant association between overall neurological status and hypocalcaemia and neither was there significant association between neurological disorder and hypocalcaemia.

Our findings seem to suggest an alarmingly high prevalence level of hypocalcaemia among our term neonates with a high occurrence of neurological disorders correlating with what has been shown in literature **(Roberton et al, 1975).** A recent retrospective study (**Cho *et al,* 2015**) found that 36% of the neonates with hypocalcaemia had tetanic spasms and increased tremulousness as the most common clinical features while **Roberton *et al,* 1975** found 17.6% of the neonates had jitteriness and irritability with a majority of the hypocalcaemic neonates not having any neurological findings. Even though this study managed to document neurological abnormalities in the hypocalcaemic neonates, we did not observe the characteristic neurological findings of neonatal hypocalcaemia as described in most literature. This could be attributed to the study design which did not provide for follow up reviews on the neonates other than a single encounter during recruitment. As a result, some of the clinical findings in the newborns could have been missed.

Overall clinically, fifty one percent of the neonates in this study had neurological disorders, 79.8% (71/89) of whom had overt convulsions. Albeit no statistically significant association was seen between neurological disorder and hypocalcaemia. However, it is imperative to note that hypocalcaemic neonates tend to have convulsions that respond poorly to anti-epileptic drugs. There is a dire need for early establishment of serum calcium levels in all neonates admitted to NICU, more so in the ones that are fitting, in order to promptly intervene with appropriate therapy. Prophylactic administration of calcium gluconate to all high risk neonates who present with convulsions has been found to be beneficial and may contribute to reduction in morbidity and duration of hospital stay of the neonates, (**Roberton *et al,* 1975)**.

The high number of hypocalcaemic and convulsing neonates seen at UTH NICU may further be explained by the high numbers of very ill neonates that are admitted to the Unit. We found that 35.6% of the neonates admitted were referred for asphyxia, 14.4% for fever, 10.3% for convulsions and 9.2% for suspected sepsis. It can be noted that these findings correlate with the final clinical diagnoses made in the neonates i.e. 33.9% had asphyxia, 33.3% sepsis and 9.8% had electrolyte imbalance. These stressful neonatal events are known to induce elevated levels of stress hormones (corticosteroids, calcitonin and glucagon) which in turn aggravate low parathyroid hormone levels leading to impaired serum calcium mobilization, (**Reynolds, 1973 and Bergman *et al*, 1974)**. In addition, these severely ill neonates (asphyxia, severe sepsis, meningitis and pneumonia) have poor or no oral intake of calcium from their mothers, they have ongoing metabolic dysregulation with resultant acidaemia thereby further increasing the risk of developing hypocalcaemia, (**Roberton *et al*, 1975).** This is supported by the results obtained from further data analysis which showed that the overall clinical diagnoses were associated with hypocalcaemia.

We also observed that the diagnosis of asphyxia and neonatal sepsis made up two thirds of the neonatal conditions seen in the study. Further on, the neonates with asphyxia had 24% increased odds for hypocalcaemia when compared to neonates with other clinical diagnoses while the neonates with sepsis had 2 times increased odds for hypocalcaemia compared to neonates with other clinical diagnoses. These findings bring to the fore that asphyxia, a preventable condition, continues to be a major cause of morbidity in NICU and hence there is need to improve antenatal and perinatal care to minimize such admissions.

Neonatal hypocalcaemia is known to be exacerbated by hypomagnesaemia, however, it was not a prominent feature in our study were only 8% of the neonates had hypomagnesaemia. There was marginal association between serum magnesium levels and hypocalcaemia although it was not statistically significant.

The mean maternal age was 24.9 years and the majority of the mothers were primi-parous. This observation unveils a finding of great significance from which important inferences can be made in view of the observed high occurrence of neonatal medical complications born to the first time young mothers. This raises pertinent questions that need answers through further research; are the neonates born from first time young mothers at increased risk of developing neonatal complications and consequently hypocalcaemia? Or perhaps is there an underlying micro-nutrient deficient status in first time young mothers that maybe having an effect on the

serum calcium levels of their newborns? Literature has shown that during pregnancy, mothers provide large amounts of calcium to the developing fetus except in instances of maternal hypo/hyperparathyroidism or vitamin D deficiency disorders, **(Kovacs, 2008)**. Vitamin D deficiency in infants and mothers is a re-emerging public health issue. In a Canadian study, they found almost half of healthy mothers (46%) and one third (36%) of their newborn term infants had plasma 25-hydroxy vitamin D levels consistent with deficiency. Risk factors associated with neonatal hypocalcaemia resulting from vitamin D deficiency which is due to maternal hypovitaminosis D include a dark skinned mother, low dietary Vitamin D maternal intake during pregnancy and lactation, and lack of maternal sun exposure, **(Shulman et al, 2008).**

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The prevalence of hypocalcemia in term neonates admitted to NICU at UTH is high with half of the hypocalcaemic neonates manifesting neurological signs such as convulsions. Only a quarter of the neonates who presented with convulsions in this study were found to have concurrent hypocalcaemia. Early identification and treatment of hypocalcaemia in the neonates is paramount in achieving prompt interventional care of severely ill term neonates who are at increased risk of electrolyte imbalance.

6.2 STUDY LIMITATIONS

Some of the limitations prominent in the study include inability to establish causality since the causes of the clinical features are multifactorial hence associations cannot be made. In addition the lack of follow up of the patients to document the full clinical picture is another major limitation as some of the evolving clinical features may have been missed.

Inability to determine the actual causes of convulsions among the neonates. There are several other causes of convulsions in neonates other than hypocalcaemia that the study was unable to account for hence it was difficult to associate convulsions with hypocalcaemia.

6.3 RECOMMENDATIONS

i. In order to reduce the length of stay of the neonates and avoid overcrowding of the NICU, early identification of the neonates with hypocalcaemia is essential. We recommend to the UTH management that measurement of serum calcium be added to the essential list of investigations.

ii. The NICU team should consider giving prophylactic calcium gluconate to all high risk neonates with potential of developing convulsions.

iii. The NICU team to conduct further research that might be able to establish the actual causes of neonatal convulsions so that evidence based NICU protocols maybe established.

REFERENCES

1. Aggarwal P et al. (1998). **Clinical predictors of outcome in hypoxic ischaemic encephalopathy in term neonates.** *Annals of tropical Paediatrics*, 18:117-121.
2. Arthur L.R, Cesare L. (1970). **A Study of Clinical, Pathological, and Electroencephalographic Features in 137 Full-term Babies with a Long-term Follow-up.** *Pediatrics* 45; 404.
3. Broner C. W, Stidham L. G, Westenkirchner D. F, Tolley E. A, (1990). **Hypermagnesaemia and Hypocalcaemia as predictors of high mortality in critically ill paediatric patients**. *Critical Care Medicine* 18 (9): 921-928.
4. Brown D. R, Steranka B. H, Taylor F.H. (1981).**Treatment of Early-Onset Neonatal Hypocalcaemia Effects on Serum Calcium and Ionized Calcium**. *American Journal of Diseases of Childhood*, 135(1):24-28.
5. Brown JK, Cockburn F, Forfar JO. (1972) **Clinical and chemical correlates in convulsions of the newborn**. *Lancet*, 1:135-139
6. Camadoo L, Tibbott R, Isaza F, (2007). **Maternal vitamin D deficiency associated with neonatal hypocalcaemic convulsions.** *Nutritional Journal*, 6:23.
7. Cho W, Yu HW, Chung HR, Yang SW, Choi CW, Kim BI, (2015). **Clinical and Laboratory Characteristics of neonatal hypocalcaemia**. *Ann Pediatr Endocrinol Metab. Journal*, 20(2):86 – 91.
8. Evelyn N. M. C, George S. R, Andrade D. M, Chow E. W. C, Silversides C. K, Bassett A. S. (2013). **Neonatal hypocalcemia, neonatal seizures, and intellectual disability in 22q11.2 deletion syndrome.**
9. Husain SM, Veligati N, Sims DG, et al (1993). **Measurement of ionized calcium concentration in neonates.** *Archives of Diseases of Childhood*, 69:77.
10. Jain A, Agarwal R, Sankar M. J, Deorari A. K, Vinod K. P, (2008). **Hypocalcaemia In The Newborn.** *Indian Journal of Paediatrics*, 75 (2): 165-169.
11. Keen JH, Lee D (1973). **Sequelae of neonatal convulsions; Study of 112 infants**. *Archives of Diseases of Childhood*, 48:542-6.
12. Kovacs CS (2008), Vitamin D in Pregnancy and Lactation: maternal, fetal, and neonatal outcomes from human and animal studies. *The American Journal of Clinical Nutrition,* 88 (suppl):520S-8S.

13. Kumar A, Gupta V, Kachhawaha J. S, Singla P. N. (1994). **Biochemical Abnormalities in Neonatal Seizures.** *Indian Paediatrics* 32: 424-428.

14. Loughead JL, Mimouni F, Tsang RC. (1988) **Serum ionized calcium concentrations in normal neonates.** American Journal Diseases of Childhood, 142:516.

15. Mehrotra P, Marwaha RK, Aneja S, Singla BM, Sharma B, (2010). **Hypovitaminosis D and Hypocalcaemic Seizures in Infancy.** *Indian Paediatrics* 47: 581-586.

16. Mimouni F, Tsang RC (1994) **Neonatal hypocalcaemia: to treat or not to treat? (A review).** *Journal of American College of Nutrition,* 13:408.

17. Mohd A. G. K, Amit U, Somashekhar C, (2010). **Efficacy of prophylactic intravenous calcium administration in first 5 days of life in high risk neonates to prevent early onset neonatal hypocalcaemia: a randomized controlled trial.** *Archives of Diseases of Childhood &Fetal Neonatal Ed*. 95: F462-F46.

18. Roberton N. R. C, Smith M. A, (1975). **Early Neonatal Hypocalcaemia.** *Archives of disease in childhood.* 50: 604 – 609.

19. Rose A. L, Lombroso C. T, (1970). **Study of Clinical, Pathological, and Electroencephalographic Features in 137 Full-term Babies with a Long-term Follow-up**. *Paediatrics* 45; 404.

20. Salle B.L, David O, Chopard J.P, Grafmeyer L.C, Kijnaud B. (1977) **Prevention of Early Neonatal Hypocalcaemia in Low Birth Weight Infants with Continuous Calcium Infusion: Effect on Serum Calcium, Phosphorus, Magnesium, and Circulating Immunoreactive Parathyroid Hormone and Calcitonin.** *Paediatric Research.* 11: 1180-1 185.

21. Shuman R, O'Gorman CS, Sochett EB, (2008). **Case 1: Neonate with Seizures and Hypocalcaemia.** *Paediatric Child Health,* 13(3): 197 – 200.

22. Thomas C. T, Smith M. J, White P. C, Adhikari S, (2012). **Transient Neonatal Hypocalcaemia: Presentation and Outcomes.** *Paediatrics* 129; e1461.

23. Tsang R, Steichen J.J, Chan G.M, (1977). **Neonatal Hypocalcaemia; Mechanism of Occurrence and Management.** *Critical care medicine*, 5; 1; 56-61.

24. Tsang R. C, William O, (1970). **Neonatal Hypocalcaemia in Low Birth Weight Infants.** *Paediatrics*, 45; 773.

25. Wandrup J, Kroner J, Pryds O, Kastrup KW (1988). **Age-related reference values for ionized calcium in the first week of life in premature and full-term neonates**. Scand Journal Clinical Laboratory Investigations, 48:255.

26. Wandrup J. (1989). **Critical Analytical and Clinical Aspects of Ionized Calcium in Neonates.** Clinical Chemistry, 35/10, 2027-2033.

27. WHO, 2011. **Guidelines on neonatal seizures.** ISBN 978 92 4 154830 4**.**

28. Zhou P, Markowitz M. (2009) **Hypocalcemia in Infants and Children.** *Pediatrics in Review* 30; 190.

APPENDICES

APPENDIX I: INFORMATION SHEET

Dear Parent/Guardian,

Introduction

We are inviting you and your baby to participate in a study. In this study, we would like to know how big the problem of low calcium (an important electrolyte for many biochemical processes in the body) in newborn babies is. This study is being done here at UTH NICU and newborn babies are being randomly selected to participate in the study.

We were prompted to do this study when we noticed that most of the newborns admitted with convulsions would not quickly respond to anti-epileptic drugs and these would eventually be found to have hypocalcaemia, a test that is not routinely done here. Hence we decided to do study to confirm these observations.

Study Title

Prevalence Of Hypocalcaemia In Term Neonates And Describe The Clinical Characteristics Of The Affected Neonates Admitted To The Neonatal Intensive Care Unit At University Teaching Hospital, Lusaka, Zambia.

Principal Investigator

Dr. Khozya D. Zyambo a post graduate student doing specialist training in Paediatrics and Child Health at the University Of Zambia School Of Medicine.

What is the purpose of the study?

The study wishes to determine how big the problem of low calcium is in newborns and what the clinical characteristics of affected newborns are. This information will help us improve our health services by providing a basis for doing routine measurements of calcium in all sick babies and intervene without delays were necessary.

What is the study procedure?

After explaining to you what the study is all about and answering any questions you may have, we will then ask you to sign a consent form agreeing to participate in the study. We will then

administer a questionnaire which will not take more than 10 minutes. Your baby will then be examined and about 1.5 to 2mls of blood will be collected during routine insertion of an intravenous line from your baby to run the three tests i.e. Calcium, Sodium and Creatinine. The blood sample will be taken to the laboratory and once the results are obtained, they will be availed to the attending clinician for appropriate action. Once the tests are run, the remaining specimen will be discarded and will not be used for any other purposes.

Your participation in this study is voluntary. Any decision made not to participate in the study will not affect the medical care of your baby. If you do decide to take part, you are free to withdraw from the study at point and this will not affect the medical care of your child. You will not be required to provide any reason for your decision to withdraw. There are no monetary incentives for participating in this study.

Possible Problems

We believe that the procedures being used in this study are not harmful to you or your baby. We do anticipate that your baby may experience pain and discomfort during collection of blood and that those babies with severe sepsis or bleeding disorders may experience delayed clotting. However, if we observe anything peculiar to you or your child before, during or after this process, we will be prompt to refer you and/or your baby for appropriate medical care to the unit doctors.

Benefits

Direct benefits to you include; free investigations, early identification of the electrolyte disorder if present, early intervention and ultimately reducing the duration of stay in hospital (early discharge). The study results will also be used to make treatment protocols for the unit and provide basis for routine measurement of serum calcium levels in all very sick babies.

Confidentiality

The investigators will endeavor to keep your personal information private. The baby's medical record will be treated just as any other hospital medical records. However, you will be given a code number (the name of the child will not be used) for identity purposes in this study. All information will be stored in a secure place (lockable cabinets with restricted access to two research assistants and the principal investigator-myself). Information from this study may be

used for other research purposes and may be published; however, your name will not be made public by the investigators**.**

Contact Details

For further information, clarifications or concerns regarding your participation in this study please use the details provided below to contact either the principal investigator or the secretary to ERES CONVERGE IRB.

Dr. Khozya D. Zyambo **(Principal Investigator)**	**The Secretary**
P/Bag Rw1x	ERES CONVERGE IRB
Department Of Paediatrics & Child Health	
University Teaching Hospital	33 Joseph Mwila Road
Lusaka, Zambia	Lusaka, Zambia.
Cell: +260977841765	Cell: +260955 155633.

PEPALA YA CHIDZIWITSO (Ci Cewa)

Pepala iyi iri ndi nkhani kapena zidziwitso zazikulu zikulu. Kulingana ndi phunziro imene tipempha inu kutengako mbali. Pafunika kuyankha mafunso ena amene mungakhale nawo pamene muganizira kutangako mbali muphunziro. Muli ndi ufulu kufunsa mafunso ku wamkulu woyanganira wofufuza (Principal investigator).

Ca Pamutu Ca Phunziro

Kupeza kapena kufufuza za kuchepekela kwa zina za izo zakuthandizila mafupa ku nkhala amphamvu (calcium) hypocalcaemia mutiana tung'ono ndi kasanthulidwe kamaonekedwe atiana wokhuzidwa ku chipatala cha tiana wosungidwa mu Neonatal Intensive Care Unit ku chipatala cha University Teaching Hospital Lusaka, Zambia.

Mkulu Wofufuza

Dotolo kapena singanga Khozya D. Zyambo ndi mwana wa sukulu amene achita ukaswiri muma phunziro yawo ku chipatala cha ana angono cho chedwa paedriatrics and child health kusukulu lalikulu ya usinganga kapena ya udotolo ku university of Zambia school of medicine.

Za Phunziro

Muphunziro iri tifuna kudziwa za kupezeka – pezeka mochepekela ya kashamu imene ithandizira mafupa ku kwima mu tiana tingono amene ku Neonatal Intensive Care Unit ndiponso mapepala wosindikizidwa maonekedwe ya tiana tokuzidwa tobadwa kwatsopano. Chidziwitso ichi chidzatithandiza kusamala kwa bwino ndi kusintha zinehitoya thupi lathanzi paku bwelesa zochitika nthawi zonse kupima molekezela kashamu mu ana onse wodwala ndi kupeza mosataya nthawi chofunika kwambiri.

Mochitila Phunziro

Tizakudziwitsani za phunziro ndi kupempha chibvomerezo pakusaina pepala yachibvomerezo iri kothela ya pepala iyi. Ndipo tidzakupatsani pepala ya mafunso mwamaola khumi – khumindizisanu motelo kuti magazi yolinga 2 mls idzatengedwa mwamasiku onse pa mizipe ya mwana wanu. Zizidziwitso zonse zolinga phunziro. Zidzasungidwa mwachisinsi Zizidziwitso ndine ndekha ndizakhala ndi ulamuliro ndi wofufuza mzanga wachiwiri ngati zopima zachitika zonse, zosalazo zizataidwa.

Zoopsya

Tikhulupira kuti zochitika ku mwana wanu muphunziro si iri yoopsya kwa inu ndi ku mwana. Tizindikira kuti mwana wanu adzamva kuwawa ndikusamvera bwino potenga magazi ndi kuti ana aja wodwalitsa kwambiri ndi ana amene amakonda kukha gazi nthawi zambiri gazi siidzaleka msanga kuchoka motelo ngati taona zoziziswa ku mwana wanu ndi inu titachita izi tidzauza mwamsanga – sanga asinganga akaswiri akuchiritsa ndi kusamalira mu unit momwemo.

Phindu

Phindu kwa inu mudza thandizika pa kulipiridwa muzopima zonse ndi kudziwa msanga mwaku chepekela zina zaizo (electrolyte). Ndikuchepesa masiku okhala muchipatala. Zotulukamo muphunziro zizathandiza kubwelesa maganizo pamodzi wa mundomeko wama chiritso mu unit ndi kubwelesa mwamasiku onse kupima ya zaizo zakukosesa mafupa (calcium) mu ana odwala kwambiri onse.

Chinsinsi

Dzina lanu sizachulidwa mugulu ya bungwe wofufuza. Mapepala amchipatala onse adzasungidwa chimodzi modzi ngati Mapepala onse amchipatala ku health centre. Nambala ya chitupa imene iwonetsa munthu aliyense kudzuwa za inu idzaonetsa chabe dongoloso ya nkhani muphunziro mwina a dongosolo kutenga nkhani pamodzi ndi kusunga mumalo yo samalika. Nkhani ku choka mu phunziro mwina a dongosolo afuna kusewezetsa njira zina ndi kulutsa koma dzina lanu siza ulutsidwa ndi bungwe ya ndongosolo.

Ngati nkotekha itatha phunziro tingathe kuona anso ku laboratale ndi kufufuza ma pepala (record) data imene ina sonkhanitsidwa nthawi ya phunziro kuthandizira mayankho kuma funso ena.

Ngati izi zachitika dzina lanu silizaulusidwa ndii adongosolo.

Njira Yowapezela

Ngati mufuna nkhani yonse ya phunziro iyi muli nayo langati wotengako mbali sewezetsani keyala iri munsi (pansipa).

APPENDIX II: CONSENT FORM

Prevalence Of Hypocalcaemia In Term Neonates and Describe the Clinical Characteristics of the affected Neonates Admitted To The Neonatal Intensive Care Unit At University Teaching Hospital, Lusaka, Zambia

Participant

I_____ **(**participant's parent or guardian's name, signature or thumb-print) have been informed about the study. I do accept voluntarily with my baby to participate in this study.

Signature/Thumb: _____ Date: _____

Interviewer

I have explained this research study to the participant and prepared to answer all queries that may arise both now or in the future regarding the study and the participant's rights.

Investigators name: _____

Signature: _____ Date: _____

Witness name: _____

Signature: _____ Date: _____

PEPALA YA CHIBVOMEREZO (Ci Cewa)

Phunziro ya kaonekedwe ndi mphimo wakuchepekera zina zaizo muma gazi mu ana angono yotandizila ku kwimisa mafupa (hypocalcaemia) ndi ku ona maonekedwe mu ana ukhuzidwa, osungidwa muchipatala cha (Neonatal Intensive Care Unit) kuchipatala cha chikulu ku University Teaching Hospital.

Wotengako Mbali

Ine ndine _____ (kholo kapena, othandiza kusunga, sainala). Ndaziwitsidwa za phunziro ndipo ndabvomera mwa ufulu kuthengako mbali ndi mwana wanga.

Chidindo chachala: _____ Tsiku: _____

Wofufuza

Ndafotokozela phunziro iri kwa otengako mbali ndidzakhala wa kuyembekezera mafunso ali wonse tsopano kapena mtsogoro. Kulingana za phunziro ndi wotengako mbali ndi ufulu wawo.

Dzina: _____ Wosaina: _____

Tsiku: _____

APPENDIX III: DATA COLLECTION SHEET

Title: Prevalence Of Hypocalcaemia In Term Neonates and the Clinical Characteristics of the affected Neonates Admitted To The Neonatal Intensive Care Unit At University Teaching Hospital.

Initials of participant:

Participant's study number:

Part I: Demographics

Mother's details:

- **a.** Age (years): _____
- **b.** Parity: _____
- **c.** Residential Address: _____
- **d.** Last Menstrual Period: _____
- **e.** HIV status: _____
- **f.** Diabetes: 1. Present 2. Absent.

Neonate's details:

- **a.** Age (days): _____
- **b.** Sex: **1. Male** **2. Female**
- **c.** Birth weight: **1. 2.5-3kg** **2. 3.1 – 3.99kg** **3. >3.99kg**
- **d.** Apgar score: At 1 minute: _____ At 5 minutes: _____ At 10 minutes: _____
- **e.** Reason for referral: _____
- **f.** Ballad score GA: **1. 37 – 40** **2. > 40**
- **g.** Gestation Age by dates: **1. 37 – 40** **2. > 40**

Part II: Clinical Findings

- **a. General appearance**: 1. Well 2. Ill
- **b. Vitals:**
  - A. Heart rate/minute: **1. < 120** **2. 120 – 160** **3. >160**
  - B. Respiratory rate/minute: **1. < 30** **2. 30 – 60** **3. > 60**
  - **C.** Temperature: **1. < 36.5** **2. 36.5 – 37.2** **3. > 37.2**
- **c. General Examination:**
  - A. Pallor: 1. Present 2. Absent

B. Jaundice: 1. Present 2. Absent

C. Cyanosis: 1. Present 2. Absent

D. Capillary refill time: 1. Normal 2. Abnormal

d. Systems/Organs

System/organ	Normal	Abnormal	Specify findings
Skin			
Heart			
Lungs			
Abdomen			
Urogenital			
Musculoskeletal			
Neurological			

Part III: Laboratory Data Sheet

a. HIV status: **1. Exposed** **2. Not-Exposed**

b. Random Blood Sugar: **1. Normal** **2. Low**

c. Serum Calcium Levels: ____ **1. Normal** **2. Low** **3. High**

d. FBC: Leucocytes:_____ Neutrophils: _____ Platelets: _____

e. Haematocrit: _____ **1. Normal** **2. Low** **3. High**

Part IV: Diagnosis; _____

Name of doctor:_____ Signature: _____

Data entry date: _____

Data entry number: _____

APPENDIX IV: BALLARD SCORE

NEUROMUSCULAR MATURITY

SIGN	SCORE							SIGN SCORE
	-1	0	1	2	3	4	5	
Posture								
Square Window	 >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 SCORE								

SIGN	SCORE							SIGN SCORE
	-1	0	1	2	3	4	5	
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 40-50mm: -1 <40mm: -2	>50 mm no crease	faint red marks	anterior transverse crease only	creases ant. 2/3	creases over entire sole		
Breast	imperceptable	barely perceptable	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								

MATURITY RATING

TOTAL 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

Gestation by Dates

	weeks

Birth date	Hour	
		am pm

APGAR	1 min	5min

Scoring

Gest. Age by Maturity Rating	_____weeks
Time of Exam	Date_____ am Hour_____ pm
Age at Exam	_____hours

Signature of Examiner

_____

_____

M.D. / R.N.

References :

Ballard JL, Khoury JC, Wedig K, *et al*: New Ballard Score, expanded to include extremely premature infants. *J Pediatrics* 1991; 119:417-423.

APPENDIX V: ETHICAL APPROVAL

33 Joseph Mwilwa Road
Rhodes Park, Lusaka
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 966 765 503
Email: eresconverge@yahoo.co.uk

I.R.B. No. 00005948
F.W.A. No. 00011697

13th October, 2014

Ref. No. 2014-June-016

The Principal Investigator
Dr. Khozya Zyambo
The University of Zambia
School of Medicine
Dept. of Paediatrics and Child Health
P.O. Box 50110,
LUSAKA.

Dear Dr. Zyambo,

RE: PREVALENCE OF HYPOGLYCAEMIA AND CLINICAL CHARACTERISTICS OF TERM NEONATES ADMITTED TO THE NEONATAL INTENSIVE CARE UNIT AT UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA.

Reference is made to your corrections dated 18th September, 2014. The IRB resolved to approve this study and your participation as principal investigator for a period of one year.

Review Type	Ordinary	Approval No. **2014-June-016**
Approval and Expiry Date	Approval Date: 13th October, 2014	Expiry Date: 12th October, 2015
Protocol Version and Date	Version-Nil	12th October, 2015
Information Sheet, Consent Forms and Dates	• English, Chichewa.	12th October, 2015
Consent form ID and Date	Version-Nil	12th October, 2015
Recruitment Materials	Nil	12th October, 2015
Other Study Documents	Data Collection Sheet	12th October, 2015
Number of participants approved for study	164	12th October, 2015

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not "stamp" approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Dr. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON