PREVALENCE OF HYPOCALCEMIA IN TERM NEONATES WITH MODERATE AND SEVERE PERINATAL ASPHYXIA IN KENYATTA NATIONAL HOSPITAL.

A dissertation submitted in part fulfillment of Masters of Medicine (M.Med) in Pediatrics and Child Health, University of Nairobi.

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H58/79726/12

MMED PEDIATRICS AND CHILD HEALTH

DECLARATION

This dissertation is my original work and hasn't been conducted in any university or published anywhere else.

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DEDICATION

This study is dedicated to all the newborns whose lives we aim to improve and to their parents.

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Prof. Wasunna and Dr. Osano for guiding and assisting me in writing this proposal. I would also like to thank Francis Njiri for his assistance while carrying out this study.

I would also like to thank my family for supporting me in this endeavor.

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LIST OF ABBREVIATIONS

AGA- Appropriate for gestational age.

ANC- Antenatal Clinic.

AMPA - Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionate.

DNA- Deoxyribonucleic Acid.

EAA- Excitatory amino acid.

FT- Full term.

IUGR- Intra uterine growth restriction.

KDHS- Kenya Demographic Health Survey.

KNH- Kenyatta National Hospital.

NBU- Newborn Unit.

NMDA- N-methyl-D-Aspartate.

PT- Preterm.

PTH- Parathyroid Hormone.

DEFINITION OF TERMS

Term neonate - Newborn infant delivered after 37 completed weeks and is less than four week old.

Hypoxic ischemic encephalopathy- A condition characterized by neurological impairment secondary to failure of adequate oxygenation and perfusion of the brain.

Hypocalcemia- A total serum calcium concentration of <2.0mmol/L in term neonates and <1.75mmol/L in preterm neonates or by the ionized fraction <1.75mmol/L in term neonates and <1.1mmol/L in preterm neonates.

Cases – Term neonates with a poor Apgar score of less than 8 at the 5^{th} minute.

Controls – Term neonates with an Apgar score of 8 and above at the 5^{th} minute.

Ante partum - Period before delivery.

Intra partum - Period during delivery.

Postnatal - The time period beginning immediately after birth and extending up to six weeks.

Apoptosis - Programmed cell death.

ABSTRACT

Introduction: Perinatal asphyxia is a common neonatal problem that contributes significantly to neonatal morbidity and mortality. It is a multi-organ disorder and some of it's' complications include cerebral palsy, organ dysfunction, hematologic disorders and metabolic disorders e.g. hyponatremia, hypocalcemia. Hypocalcemia in neonates is a major concern especially in those who have suffered perinatal asphyxia. This is because some of the clinical features of hypocalcemia e.g. jitteriness, seizures cannot be differentiated from the presentation of hypoxic ischemic encephalopathy.

Justification: Perinatal asphyxia is a common finding in our set up and it is associated with a very high mortality. Few studies have shown an occurrence of hypocalcemia in neonates with asphyxia; however, there's no local study done to determine the prevalence of hypocalcemia in neonates with asphyxia. In addition, it is vital to know exactly what an asphyxiated neonate is suffering from so as to initiate the right treatment and prevent further complications. Results from this study will help health workers make informed decisions on management of neonates with perinatal asphyxia.

Objectives: The objective was to determine the prevalence of hypocalcemia in term neonates with moderate and severe perinatal asphyxia in KNH and compare it to the prevalence of hypocalcemia in term neonates without asphyxia in KNH.

Study Setting: Newborn Unit and Maternity Ward in Kenyatta National Hospital.

Study Population: Term neonates admitted with Stage 2 or 3 encephalopathy as per Sarnat and Sarnat Staging made up the cases while the control group consisted of normal, term neonates whose gestational ages and weight were matched.

Study Procedure: Cases selected were term neonates with a poor Apgar score of less than 8 at the 5th minute who were further scored using Sarnat and Sarnat staging while controls were normal,healthy,age and weight matched term neonates from KNH maternity ward.

Methodology: The prevalence of hypocalcemia between neonates with asphyxia and normal neonates was compared using a case control study design which was carried out over a period of three months, from April to May 2016.

The cases, chosen using non randomized consecutive sampling were term neonates admitted to NBU while the controls were age and gender matched term neonates who were delivered without complications in KNH maternity. The study was carried out until the required sample size of 138 neonates was achieved.

Cases and controls were identified using a predetermined inclusion and exclusion criteria. Neonates were also staged using Sarnat and Sarnat staging criteria. Informed consent was then obtained from the mothers following which calcium blood samples were drawn. Laboratory results were availed to the primary clinicians as soon as they were ready. Neonates with hypocalcemia received calcium supplementation. Data was entered into SPSS version 20.

Standard statistical procedures were used to summarize study findings and results were presented in tables, charts and figures. Continuous variables were summarized using measures of central tendency and dispersion while categorical variables were summarized using frequency tables.

Results: The study population consisted of a total of 138 term neonates; 69 asphyxiated and 69 normal term neonates. The mean gestational age was 39 weeks with the asphyxiated term neonates having a higher mean calcium level, 2.20 compared to a mean of 2.04 found in normal neonates. All the normal term neonates were in Sarnat and Sarnat stage 0 while in the asphyxiated group, 61 (93.6%) had Sarnat and Sarnat stage 2 encephalopathy and 8 (6.4%) had stage 3 encephalopathy. In the asphyxiated group, 24 (38.1%) of the neonates had convulsions. Most of the deliveries, 99 (78.2%) were conducted at Kenyatta National Hospital with 25 (39.7%) vaginal deliveries and 38 (60.3%) cesarean deliveries in the control group and 46 (71.7%) vaginal deliveries and 7 (11.7%) cesarean deliveries in the cases group. Most of the neonates 46 (71.70%), delivered by SVD had asphyxia. The prevalence of hypocalcemia in term neonates with perinatal asphyxia was 28.8% in comparison to 1.4%, the prevalence found in term neonates without asphyxia. Term neonates with perinatal asphyxia were 10 times more likely to have hypocalcemia compared to term neonates without asphyxia. The severity of the level of asphyxia based on Sarnat and Sarnat staging is associated with a higher risk of developing hypocalcemia. In addition, the term neonates with convulsions were seven times more likely to have hypocalcemia in comparison to term neonates with no convulsions.

Conclusion: The prevalence of hypocalcemia in term neonates with perinatal asphyxia was 28.8%; with the odds of having hypocalcemia being 26.5 (95% CI of 3.4 – 206.7). In comparison, the prevalence of hypocalcemia in normal neonates was 1.4%. Obstetric factors e.g. place and mode of delivery was found to have a positive association with hypocalcemia.

Recommendations: All asphyxiated neonates should have their calcium levels monitored. Neonates with convulsions should also have their calcium levels monitored.

1.0 INTRODUCTION

Perinatal asphyxia is one of the top causes of neonatal morbidity and mortality. Globally, it was estimated in 2014 that23% of the 4 million deaths and 26% of the 3.2 million stillbirths was due to hypoxia ‹1›. According to the Kenya Demographic and Health Survey (KDHS) (2009), it was noted to cause a mortality rate of 37 deaths in 1000 pregnancies ‹2›. However, neonatal records in Kenyatta National Hospital show the mortality rate of perinatal asphyxia is at 31.1%.

Perinatal asphyxia is a common neonatal problem that contributes significantly to neonatal morbidity and mortality. It is a multi-organ disorder that results from compromised placental or pulmonary gas exchange. This disorder can lead to hypoxia and hypercarbia in the neonates' blood.

Risk factors of perinatal asphyxia can be broadly classified into:

- Ante partum conditions such as abnormal maternal oxygenation, congenital infection or anomalies, inadequate placental perfusion and/or gas exchange.
- Intra partum conditions such as interruption of umbilical circulation, inadequate placental perfusion and/or gas exchange and traumatic delivery.
- Postnatal conditions such as persistent pulmonary hypertension, severe circulatory insufficiency, congenital heart disease.

The diagnosis of perinatal asphyxia is made based on an umbilical artery blood sample pH of <7, a persistent Apgar score of 0-3 for >5 minutes, the presence of neurologic signs such as seizures or coma and the involvement of multiple organs.

The complications of perinatal asphyxia include cerebral palsy, myocardial dysfunction, renal dysfunction, pulmonary disorders, gastrointestinal dysfunction, and hematologic disorders and metabolic disorders e.g. Hyponatremia, hypocalcemia.

In perinatal asphyxia, hypocalcemia can result from:

a. Influx of calcium into the cells.
b. Decreased intake of calcium secondary to delayed feeding; early feeding in very ill neonates has been associated with ileus.
c. Renal insufficiency.

d. Increased serum calcitonin concentration.
e. Decreased Parathyroid hormone (PTH) concentration due to diminished release of the PTH.

At the biochemical level, during asphyxia, the uptake of glutamate, which is the major excitatory neurotransmitter in the brain, is impaired. This leads to high synaptic levels of glutamate and over activation of excitatory amino acid(EAA) receptors like N-methyl-D-Aspartate(NMDA), Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionate(AMPA) and kainate receptors. Activation of NMDA receptors, which are permeable to both calcium and sodium, lead to intra cellular accumulation of calcium. The difference in increased intra cellular calcium concentration and low serum calcium levels results from activation of the NMDA receptors, release of calcium from intra cellular stores and failure of calcium efflux mechanisms. This consequently leads to activation of enzymes, which cause cytoskeletal and DNA damage.

Hypocalcemia is defined as a total serum calcium concentration of <2.0 mmol/L(8 mg/dL) in term neonates and <1.75 mmol/L (7mg/dL) in preterm neonates or by the ionized fraction <1.75 mmol/L in term neonates and <1.1 mmol/L in preterm neonates ‹3›. The total calcium level measured includes the ionized, active calcium fraction and the albumin bound fraction. The ionized calcium level is affected by the albumin level, blood pH, serum phosphorous and serum magnesium.

Although most neonates with hypocalcemia are asymptomatic, the most common sign of hypocalcemia is increased neuromuscular irritability i.e. jittery and muscle jerking that is induced by environmental noise or other stimuli. Other symptoms include lethargy, abdominal distension, vomiting, wheezing and generalized or focal clonic seizures. These symptoms are non-specific as they mimic other neonatal disorders such as hypoglycemia, hypomagnesaemia, septicemia, opiate withdrawal syndrome and anoxic brain injury and thus hypocalcaemia is not easily identified.

1.2 LITERATURE REVIEW

Calcium is the most abundant mineral in the body; 99% is present in the bone while serum levels constitute <1%. Some of its functions include acting structurally as supporting material in bones, acting as second messengers in cellular signaling pathways and playing a vital role in muscle contraction and relaxation.

During pregnancy, the normal total accumulation of calcium in a fetus at term is 21g (13-33g). Approximately 80% of this accumulation occurs during the third trimester. The pregnant woman undergoes several adaptations including an increase in intestinal absorption of calcium, decreased renal excretion of calcium and increased reabsorption of calcium from the maternal skeleton. The fetal blood calcium level is maintained at a higher level (2.5-2.75mmol/L) than in the maternal circulation as the fetus establishes a particular blood calcium level, irrespective of the ambient maternal blood calcium level. This ability persists in the presence of significant maternal hypocalcemia of various causes ‹4›.

After the umbilical cord is cut and the placental calcium infusion is abruptly lost, the neonate becomes dependent on intestinal calcium intake and skeletal calcium stores to maintain a normal blood calcium level at a time of continued skeletal growth. This is reflected by the rapid fall of total and ionized over the first 6 hours of life with serum calcium levels of 2-2.25 mmol/L. Therefore, the neonate quickly turns on PTH and Vitamin D synthesis, which up regulates intestinal calcium absorption and regulates skeletal and renal handling of calcium and phosphate. There's thus gradual correction over the following 48 hours after the PTH and Vitamin D levels ascend to adult values. The normal serum calcium level in neonates is 2.12-2.57 mmol/L.

Neonatal hypocalcemia is classified by the timing of onset:

- Early Neonatal Hypocalcemia: occurs in the first 2-3 days of life and is an exaggeration of the normal decline in calcium concentration after birth. Early neonatal hypocalcemia is seen in preterm neonates, infants of diabetic mothers, neonates with intrauterine growth retardation and neonates with perinatal asphyxia.
- Late neonatal hypocalcemia: develops at the end of the 1st week of life. Can be caused by exogenous Phosphorous load, parathyroid gland failure, vitamin D deficiency and Magnesium deficiency.

Very few studies have been conducted on perinatal asphyxia and neonatal hypocalcaemia. This is especially true in Africa where only one study was carried out in Nigeria.

A prospective study done in 1993 in East, Central and Southern Africa showed the incidence of asphyxia in the newborns being 22.9% ‹5›. In 2007, a prospective hospital based study was conducted for a period of 3 weeks in the neonatal unit of Muhimbili National Hospital. A total of 362 neonates had been admitted during the time of the study in which 112 (30.9%) neonates had asphyxia. Out of these neonates, 92 had hypoxic ischemic encephalopathy and these contributed to 27.2% of the mortality rate.

In KNH, 2010, Maalim, enrolled 119 term neonates with perinatal asphyxia and carried out a hospital based short longitudinal survey where he reported the mortality rate of birth asphyxia in being 31.1% by day 7 of life ‹14›.

In Nigeria ‹7›, a case control study was carried out on 31 neonates with severe asphyxia and compared their total serum calcium concentration with 31 normal term neonates. The overall prevalence of hypocalcemia among the asphyxiated neonates was 22.6% and the asphyxiated neonates who had normal calcium levels at 12 and 24 hours, maintained normal serum levels at 48 hours of life. 57.1% of the asphyxiated neonates in this study developed convulsions although not all of them were hypocalcaemic.

Some of the limitations faced in the previous studies include the use of Apgar score in defining birth asphyxia. This scoring system, though very useful in the measurement of asphyxia, doesn't fully define birth asphyxia. In addition, other factors e.g. maternal medication may affect the Apgar score of the neonate. Onyiriuka ‹7›, in his study was unable to directly measure ionized serum calcium concentration due to lack of facilities in their hospital.

Behrman et al‹8›, in 1974 in USA compared the serum calcium levels of 42 neonates with birth asphyxia with 42 control neonates, matched for gestational age and sex. Neonates with asphyxia had lower serum calcium levels at 12 and 24 hours of life and asphyxiated neonates who received bicarbonate therapy had the least serum calcium levels.

A study done in India in 1995 by Jajoo et al‹9›, selected 35 neonates with asphyxia and 37 neonates without asphyxia and divided them into three groups; full term – appropriate for gestational age (FT-AGA) (n=30, asphyxia=15), full term – intrauterine growth restriction

(FT-IUGR) (n=20, asphyxia=10), preterm – appropriate for gestational age (PT-AGA) (n=22, asphyxia=10). Serum calcium and phosphorous levels were measured at birth, 6 hours, and 24 hours and on the 5th day of life. The asphyxiated neonates, FT-AGA as well FT-IUGR were found to have significantly lower serum calcium levels than the control infants at each of the time period studied. In contrast, the PT-AGA neonates with asphyxia were found to have significantly low serum calcium levels only on the 5th day of life. Another study done by Jain et al in 2000, compared serum total and ionized calcium levels in 25 term neonates with asphyxia and 25 normal term neonates, recruited from a university hospital in India. Asphyxiated neonates were found to have significantly lower serum total and ionized calcium levels at birth and at 48 hours of life. Low ionized calcium was detected in symptomatic neonates who had otherwise normal total calcium levels. During the study, 48% of asphyxiated neonates had abnormal clinical features.

Korkmaz et al ‹11› published a case report in 2013 on a 15 day old neonate who presented with generalized tonic clonic convulsions and apneic events associated with bradycardia and cyanosis. The baby was born at term to a 24-year-old woman following an uncomplicated pregnancy. The baby's' birth weight and length were 4050g and 52cm respectively. Physical examination revealed an active, afebrile baby with normal physical findings and facial appearance. After extensive laboratory investigations, the neonate was noted to have low serum calcium levels and hypo parathyroidism secondary to maternal hyper parathyroidism. This study summarizes that some of the clinical features of hypocalcaemia cannot be differentiated from clinical features of asphyxia.

Kisiangani‹18›, did a cross sectional study in the Maternity ward at KNH to study the prevalence and correlates of early onset neonatal hypocalcemia in term neonates. The study population consisted of pregnant women admitted in KNH and their newborn babies who were 24 to 72 hours old. Once the written consent was obtained, the mothers' blood sample was collected and 24 to 48 hours after delivery, the newborns' blood sample was collected. Neonatal hypocalcaemia was found in 21.5% of the newborns and was significantly associated with maternal hypocalcemia, which was present in 24% of the study population.

Most of the neonates with hypocalcaemia are asymptomatic. Among those who are symptomatic, the characteristic sign is increased neuromuscular irritability. These neonates often have muscle jerking and generalized or focal clonic seizures may occur. Rare

presentations include inspiratory stridor caused by laryngospasm, wheezing caused by bronchospasm or vomiting resulting from pylorospasm.

In the setting of acute hypocalcaemia, rapid treatment is necessary to prevent long-term complications. This basically consists of administering intravenous calcium gluconate and monitoring the calcium levels.

Despite previous studies, no local study has been done on hypocalcemia in neonates with perinatal asphyxia.

SUMMARY OF STUDIES ON NEONATAL HYPOCALCEMIA IN PERINATAL ASPHYXIA.

AUTHOR	RESEARCH TOPIC	STUDY DESIGN	RESULTS
R E Behrmann et al 1974 USA (8).	Neonatal hypocalcemia in infants with birth asphyxia.	Prospective randomized control trial.	Serum calcium levels were lower at 12 and 24 hours in neonates with birth asphyxia.
D Jajoo et al. 1995 India (9).	Effect of birth asphyxia on serum calcium levels.	Prospective randomized control trial.	Serum calcium levels of neonates with asphyxia measured at birth, 6 hours, 24 hours and on the 5^{th} day of life were lower compared to the controls.
A N Onyiriuka 2011 Nigeria (7).	Prevalence of neonatal hypocalcemia among full term infants with severe birth asphyxia.	Case control study.	Prevalence of neonatal hypocalcemia was 22.6%.

1.3 JUSTIFICATION

Neonatal hypocalcemia is often asymptomatic and when the clinical features are present, they tend to be non-specific. The signs and symptoms tend to mimic other neonatal disorders such as hypoglycemia, hypomagnesemia, sepsis and hypoxic ischemic encephalopathy. Untreated hypocalcemia will lead to feeding intolerance, respiratory distress and intractable convulsions.

In most centers, serum calcium is not a routine test carried out in asphyxiated neonates. Asphyxiated neonates who experience convulsions end up being treated empirically for hypocalcemia despite their levels not being known.

Hypocalcemia is one of the metabolic complications of perinatal asphyxia and its' frequency is unknown especially in our set up. Measuring calcium levels is not a routine test done on neonates with asphyxia. It is vital to anticipate and know exactly what an asphyxiated neonate is suffering from so as to initiate the correct treatment and prevent any further complications from occurring.

There's no local study done to determine the prevalence of hypocalcemia in neonates with asphyxia. Moreover, hypocalcaemia diagnosed in these neonates is more of an incidental finding. As mentioned in the introduction, the mortality rate of perinatal asphyxia in KNH is 31.1% which is very high. Perinatal asphyxia is common in our set up and this study aims to determine and compare the prevalence of hypocalcemia between term neonates with asphyxia and those without asphyxia. By highlighting the problem, it will also alert the health care workers to its occurrence.

Serum calcium concentration is an easy and affordable test which can be used as an early biochemical marker of perinatal asphyxia as it biochemically supports the clinical diagnosis and severity grading of asphyxia. This is based on the fact that severe asphyxia is characterized by intra cellular accumulation of calcium.

The results of the study can also be used to create new guidelines in managing neonates with moderate and severe perinatal asphyxia.

1.4 RESEARCH QUESTION:

What is the prevalence of hypocalcaemia in neonates with moderate and severe perinatal asphyxia in comparison to term neonates without asphyxia at Kenyatta National Hospital?

1.5 PRIMARY OBJECTIVES:

To determine and compare the prevalence of hypocalcemia between term neonates with moderate and severe perinatal asphyxia and normal term neonates at Kenyatta National hospital.

1.6 SECONDARY OBJECTIVES:

To explore maternal demographic and obstetric factors associated with hypocalcemia in term neonates with moderate and severe perinatal asphyxia and compare them to those neonates without asphyxia in Kenyatta National Hospital.

2.0 METHODOLOGY

2.1Study Design

Case control study design.

2.2 Study Setting

Kenyatta National Hospital (KNH) is the largest national and referral hospital in Kenya, it is the main inpatient hospital for the low and middle income society in Nairobi and its' environment. The cases were recruited from Newborn Unit (NBU) and the controls from the Maternity ward of KNH. The NBU admits neonates delivered in KNH and also neonates referred from other hospitals.

2.3 Study Population

The study population, both cases and controls were elected from the NBU and maternity ward in KNH. The cases selected were composed of term neonates (neonates delivered after 37 competed weeks) with a poor Apgar score of less than 7 at the 5th minute. The Apgar score was used as a tool to identify neonates at risk of asphyxia who were then staged using the Sarnat and Sarnat staging of hypoxic ischemic encephalopathy (provided below) based on the clinical assessments.

The controls selected were term neonates with a good Apgar score of more than 7 at the 5th minute, and who scored grade 0 on Sarnat and Sarnat staging. These neonates were matched with the cases in terms of gestational age and weight.

Sarnat and Sarnat Clinical Staging of Hypoxic Ischemic Encephalopathy.

	Grade I Mild	Grade II Moderate	Grade III Severe
Alertness	Hyper alert	Lethargy	Coma
Muscle Tone	Normal or Increased	Hypotonic	Flaccid
Seizures	None	Frequent	Uncommon
Pupils	Dilated, Reactive	Small, Reactive	Variable, Fixed
Respiration	Regular	Periodic	Apnoeic
Duration	<24hours	2-14 days	Weeks

2.4 Inclusion Criteria:

- Term neonates with stage 2 or 3 encephalopathy as per Sarnat and Sarnat Staging as cases.
- Normal term neonates with an Apgar score of 8 or more at the 5th minute and matched by gestational age and weight as controls.

2.5 Exclusion Criteria:

- Neonates of diabetic mothers.
- Neonates with intrauterine growth restriction.
- Neonates admitted with no record of Apgar score.

2.6 Sampling Method

The study applied consecutive sampling of admissions to the maternity ward and term neonates who satisfied the criteria were enrolled in the study between 8:00am to 6:00pm. Those who came after 6:00pm were enrolled the following morning. This was done until the required sample size of neonates was achieved.

2.7 Sample Size

The sample size was calculated using a previous estimated prevalence of a study conducted at KNH (18) in 2011 on term neonates without asphyxia which concluded that the prevalence of hypocalcaemia in normal neonates was 21.5%.

Using 22.6% as an estimate of prevalence hypocalcaemia in neonates with severe asphyxia (7), in order to detect a 3 fold increase (i.e. OR=3) in prevalence of hypocalcaemia in neonates with asphyxia when compared with normal neonates, where;

$$OR = \frac{P_1(1-P_2)}{P_2(1-P_1)}$$

This means that estimated prevalence of hypocalcaemia in asphyxia neonates will be 45.1%.

Using the formula below and assuming 95% confidence interval at 80% power:

$$n = \frac{r+1}{r}\frac{\big(P(1-P)\big)(z_\beta + z_{\alpha/2})^2}{(P_1-P_2)^2}$$

Where r = ratio of cases to controls which is equal to 1.

P =33.3%; P1 = 45.1%; P2 = 21.5%

$Z_{\beta} = 0.84$ and $Z_{\alpha/2} = 1.96$

n = 69

The sample size was thus determined as 69 for each group, a total sample size of 138.

2.8 Recruitment Procedure

I recruited patients with the help of research assistants, who consisted of clinical officers working in Pediatrics, and who I trained on procedures including how to obtain consent from the mothers and ensure confidentiality. They were also trained on physical assessments to stage neonates using Sarnat and Sarnat staging and obtaining blood samples using the broken needle method.

Neonates were staged on admission in NBU and blood samples collected within 48 hours of delivery. Blood samples were taken prior to calcium administration; the calcium levels were only known after a blood sample was collected from the patient. A finding of hypocalcemia was met with calcium supplementation; treatment was not withheld especially in an emergency setting.

2.9 Study Procedure

Once the neonates with stage 2 or 3 encephalopathy had been identified based on the level of alertness, muscle tone and presence of seizures, the study was explained to the parent or guardian and verbal consent was obtained to include the neonate in the study.

A single clinician who ensured hand hygiene and wore gloves prior to touching the neonate assessed the neonates. A swab was used to wipe the area of sample collection and a sterile needle used to collect blood from the patient. Approximately 2mls of blood was collected using the open-ended needle method, without applying a tourniquet, to avoid venous stasis and prevent artefactual hemoconcentration. Once the sample had been collected, a dry swab was placed at the site and pressure applied to minimize bleeding, the needle was discarded in a suitable sharps container.

The sample was collected in plain sample bottles and transported in an icebox to the University of Nairobi, department of pediatric laboratory, within two hours of collection. The ionized calcium concentration was analyzed using the Human kit© which utilizes a spectrophotometric method using a Lisatriol-1 series machine. Quality control procedures where standardized and control samples are run daily prior to testing the samples. Both calcium and albumin levels were analyzed to reflect the correct values of serum calcium in the neonate. Calcium levels were corrected using the equation below to reflect variances in calcium binding to albumin as the amount of total calcium varies with the level of serum albumin.

Corrected calcium = serum calcium (mmol/L) + 0.02 * (40- serum albumin (g/L).

As cases were identified, the controls were recruited from the maternity ward and matched for gestational age and weight and blood samples collected within48 hours of life.

3.0 DATA MANAGEMENT AND ANALYSIS

Data was collected using formulated questionnaires and checklists and entered into a password-protected Microsoft Access data entry platform. The entered data was assessed for completeness, accuracy and consistency before analysis was commenced. Data analysis was carried out using IBM statistics® Version 21.

Exploratory data analysis was carried out to describe the study population where categorical variables were summarized using frequency tables while continuous variables were summarized using measures of central tendency and dispersion such as mean, median, percentiles and standard deviation.

In order to determine associations between the outcome and independent variables such as gender, gestational age and weight of the neonates, level of education of the mother, economic status, and type of diet available at home; chi squared tests were used to demonstrate associations between categorical variables while parametric and non-parametric tests were used to show associations between the independent variables and continuous. In each analysis, relative risks, odds ratios, confidence intervals and p values were used to demonstrate the magnitude of the association.

To determine independent predictors of the outcome, multivariate analysis was conducted using binary logistic regression. Results are presented using tables, figures, charts and textual summaries.

4.0 RESULTS

The study was carried out from April to May 2016 and the following were recruited:

Table 1: Characteristics of the study population.

		Normal n = 69	Asphyxiated n = 69	Total n = 138
Gestation (weeks)	Mean	39	39	39
	Standard Deviation	1	1	1
Birth Weight (g)	Mean	3167.55	3156.38	3161.96
	Standard Deviation	390.07	542.00	470.49
Length (cm)	Mean	46.25	48.20	47.22
	Standard Deviation	2.62	5.34	4.30
Head Circumference (cm)	Mean	34.33	35.15	34.73
	Standard Deviation	1.36	2.98	2.33
Gender	Male	33	41	74
	Female	36	28	64

The study population consisted of 138 neonates, 69 of who had asphyxia while the rest were normal term neonates. The neonates recruited into the study had a mean gestational age of 39 weeks. Both cases and controls had an almost similar mean birth weight of approximately 3,100g. The study population consisted of 74 (55.20%) male term neonates and 64 (44.80%) female term neonates, of note the cases had a higher number of males (41) than females (28), while the control group was relatively evenly matched.

Table 2: Severity of asphyxia in the study population.

		Normal		**Asphyxiated**		**Total**	
		n	**%**	**n**	**%**	**n**	**%**
Sarnat Stage	2	0	0.00	61	93.70	61	46.80
	3	0	0.00	8	6.30	8	3.20
Convulsions	No	69	100.00	41	61.90	110	81.00
	Yes	0	0.00	28	38.10	28	19.00

All the non-asphyxiated neonates were in Sarnat stage 0. In the asphyxiated group, 61 (93.7%) had stage 2 encephalopathy while 8 (6.30%) had stage 3 encephalopathy. As was expected, none of the non-asphyxiated neonates had convulsions while 28 (38.1%) of the asphyxiated neonates had convulsions.

Table 3: Maternal demographic characteristics.

Characteristics		Normal		Asphyxiated		Total		P value
		n	%	n	%	n	%	
Marital status	Single	8	11.80	16	23.90	24	17.80	0.029
	Married	55	87.30	47	70.10	107	79.30	
	Separated	0	0.00	4	6.00	4	3.00	
Occupation	Salaried formal	20	29.00	15	22.10	35	25.55	<0.0001
	Informal	4	5.80	18	26.80	22	16.10	
	Self employed	17	24.60	22	32.40	39	28.50	
	Unemployed	28	40.60	13	19.10	41	29.90	
Education level	None	1	1.40	0	0.00	1	0.70	0.123
	Primary	8	11.60	12	17.90	20	14.70	
	Primary not completed	1	1.40	2	3.00	3	2.20	
	Secondary	15	21.70	26	38.80	41	30.10	
	Secondary not completed	13	18.80	11	16.40	24	17.60	
	Tertiary	31	44.90	16	23.90	47	34.60	

Most of the mothers, 107 (79.30%) were married and 41 (29.90%) of the mothers were unemployed while the rest were under various sectors of employment. The level of employment was associated with asphyxia with more mothers who were self-employed 22 (32.40%) delivering neonates with asphyxia (P value <0.0001). The marital status and level of education did not have any significant impact on delivering neonates with asphyxia.

Table 4: Maternal obstetric characteristics.

Characteristics		Normal		Asphyxiated		Total		P value
		n	%	n	%	n	%	
Parity	1	32	46.40	30	44.10	62	45.30	0.078
	2-4	36	52.20	31	45.60	67	48.90	
	>4	1	1.40	7	10.30	8	5.80	
ANC visits	No	4	4.40	2	1.50	6	3.20	0.309
	Yes	65	95.20	67	98.40	132	96.80	
Calcium supplementation in the mother	No	55	79.70	69	100.00	124	89.60	<0.0001
	Yes	14	20.30	0	0.00	14	10.40	
Food taboos	No	60	87.00	65	97.00	125	91.10	0.031
	Yes	9	13.00	4	3.30	13	8.90	
Place of delivery	Clinic	0	0.00	30	43.30	30	21.30	<0.0001
	Hospital	69	100.00	39	56.70	108	78.70	
Mode of delivery	SVD	29	42.00	50	71.20	79	56.30	<0.0001
	Breech delivery	0	0.00	3	4.50	3	2.20	
	Assisted delivery	0	0.00	8	12.10	8	5.90	
	CS	40	58.00	8	12.10	48	35.60	
Duration of labour	<12 hours	44	63.80	50	71.20	94	67.40	0.241
	>12 hours	25	36.20	19	28.80	44	32.60	

Most 132(96.80%) of the mothers attended the antenatal clinics more than 4 times but 124 (89.60%) of the mothers were not on any form of calcium supplements. This was significantly associated with asphyxia (P value of <0.0001).Out of the total 138, 108 (78.70%) of the neonates were delivered in hospital while the rest were delivered in the

clinics. The place and mode of delivery were associated with asphyxia (P value <0.0001). Food which was considered taboo to 13 (8.90%) mothers consisted of kale, eggs, beans and yoghurt. Use of calcium supplementation in the mothers, the place and mode of delivery were the only obstetric factors associated with asphyxia.

Table 5: Laboratory results.

		Normal	**Asphyxiated**	**Total**
Calcium	**Mean**	2.04	2.20	2.12
	Standard Deviation	0.31	3.46	2.42
Albumin	**Mean**	43.97	40.66	42.35
	Standard Deviation	4.02	7.14	5.97

The mean ionised calcium levels in the non-asphyxiated neonates was 2.04 mmol/Lasphyxiated neonates had a higher mean calcium level of 2.20mmol/L. Albumin levels were also measured and neonates with hypoalbuminemia had their calcium levels corrected using the formula mentioned previously.

Table 6: Prevalence of hypocalcemia.

	Asphyxia	**Normal**
Hypocalcemia	19	1
Normal calcium levels	50	68
Total	69	69

While 19 asphyxiated neonates had hypocalcemia, only 1 normal term neonate was found to have a low calcium level.

Table 7: Factors associated with hypocalcaemia.

Characteristics		Hypocalcaemia		Normal		OR(95% CI)	P value
		n	%	n	%		
Presence of asphyxia	Asphyxia	19	28.80	50	71.20	26.50(3.4 -206.7)	<0.0001
	Non-asphyxia	1	1.40	68	98.60	1	
Gender	Male	14	19.70	58	80.30	2.6(0.9-7.9)	0.074
	Female	6	9.50	60	90.50	1	
Sarnat stage	1	1	1.40	68	98.60	1	<0.0001
	2	16	27.60	45	72.40	24(3.2-194.4)	
	3	3	37.50	5	62.50	62(3.8-1002)	
Convulsions	Yes	14	50.00	14	50.00	18.3(5.8-57.9)	<0.0001
	No	6	5.60	104	94.40	1	
Calcium supplementation in the mother	No	19	15.80	105	84.20	1	0.212
	Yes	0	0.00	14	100.00	1	
Place of delivery	Clinic	8	27.60	21	72.40	3.9(1.4-11.6)	0.008
	Hospital	10	9.60	94	90.40	1	
Mode of delivery	SVD	15	20.00	60	80.00	5.7(1.2-26.4)	0.015
	Breech	0	0.00	2	100.00		0.358
	Assisted delivery	1	14.30	6	85.70	3.6(0.3-45.8)	
	CS	2	4.20	46	95.80	1	
Duration of labour	<12 hours	14	15.90	80	84.10	2.5(0.7-9.3)	0.158
	>12 hours	4	9.10	40	90.90	1	

The prevalence of hypocalcemia in term neonates with asphyxia was 28.80% while the prevalence of hypocalcemia in term neonates without asphyxia was 1.40%. The odds of neonates with asphyxia having hypocalcemia was 26.5 (95% CI of 3.4-206.7) higher

compared to term neonates without asphyxia. For neonates with Sarnat and Sarnat staging, stage 3 encephalopathy, the odds of developing hypocalcemia was 62 (95% CI of 3.8-1002) times higher than in neonates in Sarnat and Sarnat stage 0 encephalopathy. Presence of asphyxia, convulsions and the level of Sarnat stage was associated with hypocalcemia (P value <0.0001).

In assessing maternal factors, the place and mode of delivery were risk factors for asphyxia and subsequent hypocalcemia.

A backward binary logistic regression was carried out to identify independent factors associated with hypocalcemia; starting with a full model which included all variables that had been significant during bivariate analysis.

Table 8: Multivariate Analysis.

	Coefficient	S.E. of coefficient	P value	OR (95% CI)
Asphyxia	2.332	1.116	.037	10.303 (1.157-91.77)
Convulsions	1.887	.611	.002	6.600 (1.993-21.853)

Term neonates with asphyxia were ten times more likely to have hypocalcemia as compared to term neonates without asphyxia. In addition, neonates with convulsions were 7 times more likely to have hypocalcemia in comparison to neonates with no history of convulsions. Due to the small sample size, the confidence intervals were notably wide.

5.0 DISCUSSION

Hypocalcemia in neonates is a significant problem especially in those with perinatal asphyxia. This study aimed to get the prevalence of hypocalcemia in term neonates with asphyxia and compare it to term neonates without asphyxia and look at the associated factors. Based on the case control formula used to calculate the sample size, the initial sample size consisted of more than 2000 neonates. Due to financial and time constraints, 22.6% was used as an estimate of prevalence of hypocalcaemia in neonates with severe asphyxia (18) and a total sample size of 138 was arrived at.

In this comparative study, the prevalence of hypocalcemia in neonates with asphyxia was 28.80% while in term neonates without asphyxia, the prevalence was 1.40%.

Several studies have been conducted; one in 1974 in USA (8) and another study done in 1995 in India (9) that found neonates with asphyxia had hypocalcemia compared to neonates without asphyxia. The hypocalcemia was detected as early as 12 hours post-delivery and persisted up to day 5 of life. There have been very few studies, which assessed hypocalcemia in neonates with asphyxia in developing countries.

A study carried out in 2011 in Nigeria (7) looked at the prevalence of hypocalcemia in neonates with severe asphyxia. The study used Apgar score to classify the degree of asphyxia and measured total calcium levels. The overall prevalence of hypocalcemia among the severely asphyxiated neonates was 22.6% and the asphyxiated neonates who had normal calcium levels at 12 and 24 hours, maintained normal serum levels at 48 hours of life. According to this study, 57.1% of the asphyxiated neonates developed convulsions although not all were hypo calcemic; it wasn't clear how many of the neonates convulsed from hypocalcemia and how many convulsed from the encephalopathy.

This is in comparison to a study carried out by Kisiangani at KNH in 2011 (18) which found an overall prevalence of neonatal and maternal hypocalcemia to be 21.50% in normal term neonates and 24% in the mothers respectively. In his study, maternal hypocalcemia was significantly associated with neonatal hypocalcemia and hypocalcemia was associated with neonates whose birth weights were lower than those with normal calcium levels. The low

prevalence of hypocalcemia in normal term neonates found in this study could be attributed to a low prevalence of maternal hypocalcemia, which unfortunately was not assessed.

The prevalence of hypocalcemia in neonates with asphyxia was higher (28.8%) compared to the study carried out by Onyiriuka (22.6%) as his study focused on neonates with severe birth asphyxia only; while this study determined the prevalence of hypocalcemia in both moderate and severe perinatal asphyxia.

The level of consciousness, muscle tone, reflexes and presence of convulsions were used to classify which stage of Sarnat the neonates fell in. A neonate had to have at least 3 of the characteristics to be classified into a specific stage.

All the non-asphyxiated neonates were in Sarnat stage 0. In the asphyxiated group, 61 (93.70%) of the 69 asphyxiated neonates had stage 2 encephalopathy while 8 (6.40%) had stage 3 encephalopathy. Out of the total 138 neonates, 28 (38.10%) had convulsions and they all had asphyxia. In the asphyxiated group, a total of 19 neonates had hypocalcemia, 14 (20.63%) of who developed convulsions. The other 5 asphyxiated neonates who had convulsions had normal calcium levels; the convulsions were assumed to be due to the encephalopathy. This is in comparison to the study carried out in Nigeria (7), where 57.10% of the asphyxiated neonates developed convulsions. However, it was not clear what percentage of these neonates who convulsed had hypocalcemia.

From the results of the study, the presence of asphyxia was found to be associated with hypocalcemia as 28.8% of the asphyxiated neonates had hypocalcemia and neonates with asphyxia had 26.5 odds of hypocalcemia as compared to the neonates without asphyxia. In addition, the level of Sarnat stage is also closely associated with the development of hypocalcemia with neonates with stage 3 encephalopathy having a higher risk of hypocalcemia. The prevalence of hypocalcemia in stage 2 encephalopathy was 28.6% while that of stage 3 encephalopathy was 50.0%. This is consistent with the findings made in previous studies on neonatal hypocalcemia in infants with asphyxia (8).The level of hypocalcemia tends to remain constant over a period of days as seen in the study carried out in India (9) so treatment has to be initiated once hypocalcemia is detected.

Convulsions were noted to be associated with hypocalcemia with 14 (54.20%) of the neonates who had convulsions having hypocalcemia. While metabolic disturbances are

known to cause convulsions, hypoxic ischemia is still the leading cause of neonatal seizures (20) and is associated with a poor long-term outcome (21).

Several maternal factors were associated with neonatal hypocalcemia. These included mode of delivery with neonates being delivered via spontaneous vertex delivery having a higher risk of asphyxia and hypocalcemia. More neonates were delivered in the hospital than in the clinics and these neonates had a poorer outcome than those delivered in the clinics. This could be explained by the fact that this study was carried out during a period when the nursing staff at Pumwani Maternity Hospital, a major delivery hospital for many women in Nairobi, had gone on strike. This led to an extremely high rate of admission in the maternity ward in KNH, which led to overwhelming of the health staff and impaired service delivery. In addition, high risk mothers are usually referred to hospitals for delivery.

Use of calcium supplements by the mother was not found to be a risk factor for neonatal hypocalcemia. Out of the total 138 mothers, only 14 mothers were on calcium supplements. However, calcium supplementation by the mothers was associated with asphyxia. It's important to note that the 14 women on calcium supplementation delivered normal, term, non-asphyxiated neonates.

Another factor that didn't have any statistical significance was the practice of food taboos, which wasn't very common in the study population. Food forbidden to the 13.0% of the mothers included eggs, kale, yoghurt and beans, food which is considered in many cultures to cause hyperacidity and gastritis in pregnant women. These taboos are basically based on the fact that they cause discomfort to the pregnant woman.

5.1 LIMITATIONS.

Due to financial and time constraints, the calculation of sample size had to be modified using an odds ratio. As a result a much smaller sample size was used and this led to a wide confidence interval in the variables.

Hormonal assays, maternal parathyroid hormone levels in particular and maternal levels of calcium were also not done due to financial constraints. This would have further shown an association between maternal and neonatal levels of calcium.

5.2 CONCLUSION.

1. The prevalence of hypocalcemia in neonates with asphyxia is higher (28.8%) than in term neonates without asphyxia (1.4%).
2. Factors associated with hypocalcemia include the place and mode of delivery.

5.3 RECOMMENDATIONS.

1. All asphyxiated neonates and neonates with convulsions should have their calcium levels monitored.
2. A larger study can be carried out which may yield more conclusive findings.

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APPENDICES

Appendix I: Informed consent

CONSENT FORM.

Patient study identification number.....**Date**.....

Study title: Prevalence of hypocalcemia: a comparison study in term neonates with moderate and severe perinatal asphyxia and term neonates without asphyxia in KNH.

Investigator: Dr Mohamed (Registrar in Department of Paediatrics and Child Health at University of Nairobi).

Introduction: A study is being carried out in KNH, by a registrar studying Masters degree in Pediatrics and Child Health, to assess and compare the levels of calcium in babies who were born normal and babies born with asphyxia (asphyxia is a condition that happens when a babys' brain and body does not get enough oxygen).The study is voluntary and there will be no financial rewards. This study which is in part fulfillment of the Masters programme, will involve collecting blood samples from the neonates.

i. **Risks**.

The study will carry minimal risk to your child. A small sample of blood will be required from your baby to assess the levels of calcium and albumin. Treatment will not be withheld and refusal to participate in the study will not alter the management that your baby will receive while at the hospital.

ii. **Benefits**.

During the study you will be advised on how to take care of your baby and identify any danger symptoms. The results will be used by the healthcare providers in this hospital to help improve care of babies with asphyxia. In addition, yo will not incurr any additional costs.

iii. Confidentiality.

You as the participant in the study will be identified by use of a code and not by name. The information obtained will be kept in strict confidence and not be released to any other person without your permission.The overall findings obtained will be made available to the relevant teams involved in the management of babies with asphyxia. You are free to withdraw from the study any time.

If you have any concern or question, kindly contact the investigator Dr Mohamed by calling 0723-311339.

I .....as.....to baby.....having received information on the study, benefits and risks hereby AGREE/DISAGREE (cross out as appropriate)to participate in the study with my baby.I understand that participation is voluntary.
Parent/ guardian's signature.....date.....

I .....declare that I have adequately explained the information to the parent/guardian on the study, benefits, risks and given him/her time to ask questions and seek clarification regarding the study. I have answered all the questions to the best of my ability.
Researcher/ Research assistants'
signature.....date.....

In case of any questions, the following can be contacted:

KNH – UoN Ethical Review Secretariat,
P.O.BOX 20723-00202,
Nairobi.
Tel: 726300-9.

Supervisor: Prof Wasunna
0722700444.

FOMU YA IDHINI.

NAMBARI YA MGONJWA----------TAREHE ----------

SWALA KUU LA UTAFITI: Kiwango cha calcium kati ya watoto waliozaliwa na upungufu wa oxygen na watoto waliozaliwa bila upungufu wa calcium

Mtafiti: Daktari Mohamed (Daktari Katika Kitengo cha Watoto katika Chuo kikuu cha Nairobi).

Introduction: Kuna tokeo linaloendelea hapa kwenye hospitali kuu ya Kenyatta lenye kuangalia na kupima kiwango cha calcium katika watoto wachanga waliozaliwa na upungufu wa oxygen. Utafiti huu ni wa kujitolea na hakuna ahadi ya wewe kunufaika kifedha. Tuna matumaini kuwa matokeo ya utafiti huu, ambao unahitaji mtoto kutolewa damu mara moja, yatanufaisha madaktari wakati wanawatibu watoto walizaliwa na upugufu wa hewa.

i. **Madhara**.

Utafiti huu hauna madhara yeyote kwa mtoto ambaye atatolewa damu kidogo kwa sababu ya utafiti wa kupima calcium. Iwapo utakataa kushiriki, hili halitabadilisha matibabu ya mtoto wakati yupo kwa hospitali.

ii. **Manufaa**.

Utapokea mawaidha kuhusu uchungaji wa mtoto na jinsi ya kutambua ugonjwa kwa mtoto. Kama mzazi wa mtoto anayehusika na tokeo hili, hutatozwa pesa kwa mtoto kupimwa calcium. Matokea ya utafiti yatakuwa ya manufaa kwa washikadau na wafanyikazi katika Kitengo cha afya haswa kwa kuimarisha matibabu ya watoto wengine.

iii. **Ya siri**.

Wewe kama mhusika, utajulikana kwa nambari tu na sio kwa jina lako au lile la mtoto. Majibu ya utafiti yatabaki kuwa siri na hayataruhusiwa kuonekana na mtu mwingine bila ruhusa yako. Matokeo ya utafiti kwa jumla yatapewa washikadau ambao wanahusika na mipango na matibabu ya watoto wachanga lakini hayatuzungumzia mtoto wako kibinafsi.

Kama uko na swali lolote, wasiliana na mtafiti mkuu; Daktari Mohamed, nambari ya simu 0723311339.

Mimi .....kama.....nimeelewa maana na jinsi wa utafiti huu, na nimepeana idhini baada ya kuelezwa kuhusu madhara na manufaa yake.

NIMEKUBALI/NIMEKATAA (futa moja ya haya mawili) kushiriki katika utafiti huu na ninafahamu kuwa ni wa kujitolea na nina uhuru wa kujiondoa.

Sahihi.....Tarehe .....

Mimi .....natangaza kuwa nimepeana habari ya utafiti huu kwa mzazi wa mtoto haswa kuhusu madhara na manufaa na nimekubali kuulizwa maswali na nimeyajibu kwa uwezo wangu wote.

Sahihi ya mtafiti.....Tarehe.....

Kwa maswali yeyote, unaweza kuuliza:

Mwenyekiti,
KNH – UoN Ethical Review Secretariat,
P.O.BOX 20723-00202,Nairobi.
Tel: 726300-9.

Au:

Prof. Wasunna,
0722700444

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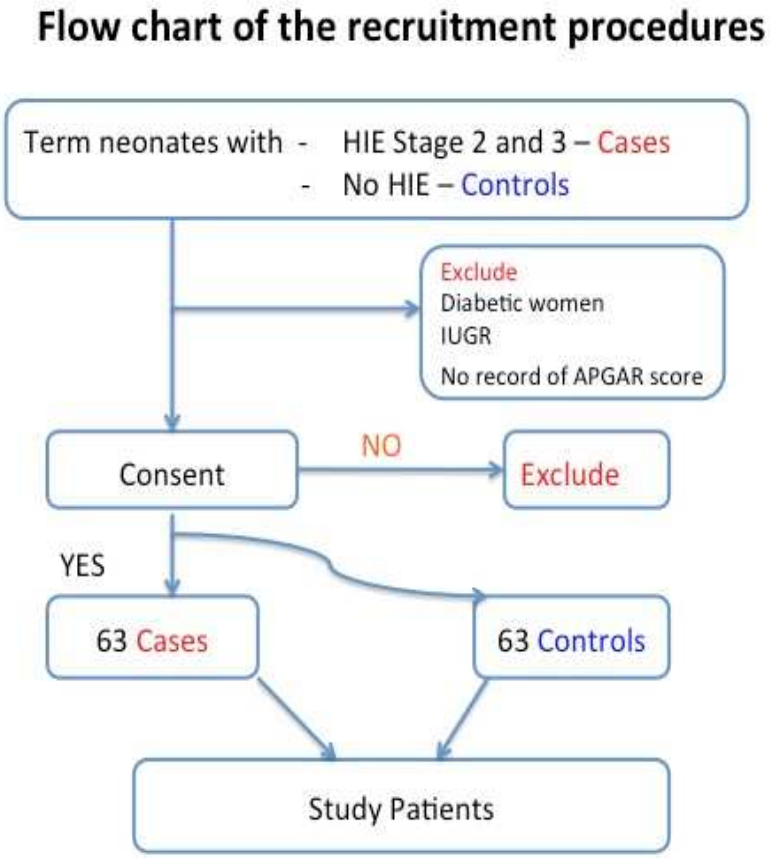
Appendix II: Screening and Recruitment process flow chart



Appendix III: Data Collection Tools.

QUESTIONNAIRE

Patients Study Identification Number..... Hospital No..... Date.....

Patients Data		
Gender	(0) Male	(1) Female
Date of birth		
Time of admission		
Gestational age in weeks		
Birth weight (g)		
Length (cm)		
Head circumference (cm)		
Sarnat and Sarnat Clinical Staging of HIE		
Level of consciousness (2) Alert, (1) Lethargic, (0) Coma		
Muscle tone (2) Normal, (1) Hypotonic, (0) Flaccid		
Reflexes (2) Normal/Exaggerated, (1) Weak, (0) Absent		

Mothers' Data		
Date of birth		___/___/___
Parity		(1) 1, (2) 1-4, (3) >4
Marital status		(1) Single, (2) Married, (3) Separated, (4) Widowed.
Occupation		(1) Salaried formal, (2) Informal, (3) Self employ, (4) Unemployed
Level of education		(1) None, (2) Primary, (3) Primary not completed, (4) Secondary, (5) Secondary not completed, (6) Tertiary.
ANC Visits	Don't know	(1) No, (2) Yes
Calcium supplements during pregnancy	Don't know	(1) No, (2) Yes
Food taboos	Don't know	(1) No, (2) Yes Specify_____
Place of delivery		(1) Home, (2) On the way to the hospital, (3) Clinic, (4) Hospital.
Mode of delivery		(1) SVD, (2) Breech delivery, (3) Assisted delivery, (4) CS.
Duration of labor	Don't know	(1) <12hrs, (2) >12hrs.

CLINICAL MANIFESTATIONS IN NEONATES

Clinical Features	Neonates with Perinatal Asphyxia and Hypocalcemia.	Neonates with Perinatal Asphyxia without Hypocalcemia.	Normal neonates with Hypocalcemia.
Lethargy			
Jitteriness			
High pitched Cry			
Hypertonia			
Twitching			
Convulsions			

LABORATORY RESULTS

	HYPOCALCEMIA	NORMAL CALCIUM LEVELS*
NEONATES WITH ASPHYXIA		
NORMAL TERM NEONATES		

*Normal reference ranges for total serum calcium in neonates is 2.12-2.57mmol/L; 1.75 – 2.2mmol/L for ionized calcium levels.

Appendix IV: Budget.

Budget

ITEM	QUANTITY	UNIT PRICE	TOTAL (KSH)
SUPPLIES			
Biro Pens	6	20.00	120.00
Pencils	6	10.00	60.00
Box file	3	150.00	450.00
Spring files	3	100.00	300.00
Pencils sharpener	1	50.00	50.00
White out pen	1	150.00	150.00
Folder	4	50.00	200.00
Staple	1	500.00	500.00
Paper Punch	1	600.00	600.00
Staple Remover	1	250.00	250.00
Note book	1	100.00	100.00
TOTAL SUPPLIES		**1930.00**	**2780.00**
OTHERS			
Printing and Photocopying		10,000.00	10,000.00
Final proposal booklet	5	4,000.00	20,000.00
Ethics Committee Book	1	2,000.00	2,000.00
A poster	1	2,000.00	2,000.00
TOTAL OTHER		**18,000.00**	**34,000.00**
Blood Tests			**162,000.00**
Communication			**10,000.00**
Statistician fee			**20,000.00**
TOTAL PERSONNEL			**192,000.00**
TOTAL EXPENSES			**228,780.00**

Appendix V: LETTER OF APPROVAL FROM KNH/UON-ERC

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/186

22nd April, 2015

Dr. Nayirat Mohamed
H58/79726/2012
Dept. of Pediatrics and Child Health
School of Medicine
University of Nairobi

Dear Dr. Mohamed

Research Proposal : Prevalence of hypocalcemia in term neonates with moderate and severe perinatal asphyxia in comparison to term neonates without asphyxia in Kenyatta National Hospital (P735/12/2014)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 22nd April 2015 to 21st April 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chair, KNH/UoN-ERC
The Dean, School of Medicine, UoN
The Chair, Dept. of Pediatrics and Child Health, UoN
Supervisors: Prof. A. Wasunna, Dr. B. Osano