PREVALENCE OF METABOLIC BONE DISEASE AMONG PRETERM INFANTS USING BIOCHEMICAL MARKERS AT 6 WEEKS POST NATAL AGE AT KENYATTA NATIONAL HOSPITAL

Dr. Wambui Wanyoike (MBChB, UON)
H58 / 80712 / 2015

A Dissertation in Part Fulfilment of the Requirements for the Degree of Masters of Medicine in Paediatrics and Child Health, University of Nairobi.

2018
DECLARATION

Student Declaration
I declare that this dissertation is my original work and has not been presented in any other university or institution for the award of a degree or any academic credit.

Dr. Wambui Wanyoike
Registrar, Department of Paediatrics and Child Health
University of Nairobi
School of Medicine
SIGNED …………………………… Date ……………………………

Supervisor’s Declaration
This dissertation has been submitted with our approval as university supervisors.

Professor R. N. Musoke
Professor of Paediatrics and Child Health,
Lecturer, Department of Paediatrics and Child Health,
University of Nairobi
SIGNED …………………………… Date ……………………………
Dr. Rashmi Kumar
Senior Lecturer, Department of Paediatrics and Child health
University of Nairobi
SIGNED …………………………… Date ……………………………

Dr. Lucy Mungai
Lecturer, Department of Paediatrics and Child health
University of Nairobi
SIGNED …………………………… Date ……………………………
DEDICATION
This work is dedicated to my dearest parents, Anne and Mohammed Wanyoike, my loving siblings, Mburu and Muthoni and my new born son. To my grandmother and friends. Thank you all for your encouragement, support and love.
ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to

1. To God for holding my hand throughout the process

2. My supervisors, Professor Musoke, Dr. Kumar and Dr. Mungai, for their mentorship and guidance.

3. My research assistant, Prisca, thank you for always being available and helpful with data collection.

4. Dr. Hussein Dosajee, for his assistance in data analysis and management.

5. The staff of Kenyatta National Hospital, New Born Unit and outpatient clinics, and the records department for their support and facilitating smooth running of data collection.

6. All the mothers of the newborns who participated in this study.
ABBREVIATIONS

ALP: Alkaline phosphatase
APA: Appropriate for gestational age
BMC: Bone mineral content
BMD: Bone mineral density
ELBW: Extreme low birth weight
G: Gram
GA: Gestational age
GH: Growth hormone
HC: Head circumference
IU: International Units
IUGR: Intrauterine growth restriction
LBW: Low birth weight
LNMP: Last normal menstrual period
MBD: Metabolic bone disease
MD: Mean difference
Mg: Milligram
NEC: Necrotizing enterocolitis
OR: Odds ratio
PTHrP: Parathyroid-hormone related peptide
RCT: Randomized control trial
ROP: Rickets of prematurity
SD: Standard deviation
SGA: Small for gestational age
TPN: Total Parenteral Nutrition
TRP: Tubular reabsorption of Phosphorus
UoN: University of Nairobi
VLBW: Very low birth weight
WHO: World Health Organization
DEFINITIONS OF TERMS

Metabolic bone disease: decrease in the bone mineral content relative to the expected level of mineralization for a fetus or infant of similar size or gestational age together with biochemical and/or radiographic changes (1).

Preterm: Infant born before 37 completed weeks of gestation

Low birth weight: Birth weight of 1500-2000 grams.

Very low birth weight: Birth weight of 1000-1499 grams.

Extreme low birth weight: Birth weight of less than 1000 grams.

Bone mineral content: Measurement of the quantity of minerals in a bone segment.

Bone mineral density: Quantity of minerals contained in a certain volume of bone.

Newborn: a baby under 28 days of life

Tubular reabsorption of phosphorus: Measure of the fraction of filtered phosphate that is reabsorbed.
# TABLE OF CONTENTS

DECLARATION.............................................................................................................ii  
DEDICATION.................................................................................................................iii  
ACKNOWLEDGEMENTS.................................................................................................iv  
ABBREVIATIONS.........................................................................................................v  
DEFINITIONS OF TERMS..............................................................................................vi  
TABLE OF CONTENTS .................................................................................................vii  
LIST OF TABLES .............................................................................................................ix  
LIST OF FIGURES ..........................................................................................................x  
ABSTRACT .....................................................................................................................xi  

1. INTRODUCTION AND LITERATURE REVIEW .............................................. 1  
   1.1 Introduction ........................................................................................................ 1  
   1.3 Risk Factors for MBD ...................................................................................... 6  
   1.4 Screening and Diagnosis of MBD of Pre-terms ............................................. 7  
   1.5 Treatment and Prevention of MBD ................................................................. 10  
   1.6 Complications of MBD of Preterms ............................................................... 11  

2. STUDY JUSTIFICATION AND UTILITY ...................................................... 12  

3. STUDY OBJECTIVES ......................................................................................... 14  
   4.1 Study Design .................................................................................................. 15  
   4.2 Study Area ..................................................................................................... 15  
   4.3 Study Population............................................................................................ 15  
   4.4 Sample Size Calculation ............................................................................... 16  
   4.5 Sampling Methods ....................................................................................... 17  
   4.6 Study Variables ............................................................................................. 17  
   4.7 Study Tools ................................................................................................... 17  
   4.8 Study Period ................................................................................................ 17  
   4.9 Selection and Enrolment of Participants and Consent Procedure ............. 17  
   4.10 Study Procedures ....................................................................................... 18  

5. DATA COLLECTION, MANAGEMENT AND ANALYSIS .......................... 21  
   5.1 Data Collection ............................................................................................. 21  
   5.2 Data Management and Analysis .................................................................. 21  
   5.3 Study Limitations ......................................................................................... 22  
   5.4 Study Closure and Procedure ..................................................................... 22  

6. ETHICAL CONSIDERATIONS .............................................................................. 23
7. RESULTS .......................................................................................................................... 25
  7.1 Sociodemographic Characteristics ............................................................................. 26
  7.2 Disease Prevalence ..................................................................................................... 27
  7.3 Growth Parameters .................................................................................................... 30
  7.4 Feeding Patterns ........................................................................................................ 31
  7.5 Risk Factors .............................................................................................................. 31
  7.6 Biochemical Parameters ............................................................................................ 33
8. DISCUSSION ..................................................................................................................... 35
9. CONCLUSIONS AND RECOMMENDATIONS ............................................................. 38
  9.1 Conclusions ................................................................................................................ 38
  9.2 Recommendations ..................................................................................................... 38
10. REFERENCES .................................................................................................................. 39
    Appendix 1: Consent Information Form ........................................................................ 43
    Appendix 2: Data collection form .................................................................................. 50
    Appendix 3: Study Budget ............................................................................................. 53
    Appendix 4: Time Frame ............................................................................................... 55
LIST OF TABLES

Table 1: Patient Characteristics and Anthropometric Measurements .................. 27
Table 2: Frequency of MBD between the different age groups............................ 28
Table 3: Frequency of MBD in relation to birth weight .................................... 29
Table 4: Distribution of MBD based on Intrauterine Growth Assessment ............ 29
Table 5: Growth Parameters at 6 weeks ............................................................ 30
Table 6: Growth increments in neonates with and without MBD ....................... 30
Table 7: Feeding Patterns and MBD ................................................................. 31
Table 8: Bivariate Analysis of risk factors vs presence of MBD ......................... 32
Table 9: Proportion of MBD in neonates with the risk factors ......................... 32
Table 10: Calcium and Phosphorus levels in the sample population ................. 33
Table 11: Calcium levels in neonates with and without MBD ............................ 34
LIST OF FIGURES

Figure 1: Patient recruitment and follow up flow chart ........................................ 25
Figure 2: Gender distribution .................................................................................. 26
Figure 3: Prevalence of Metabolic Bone Disease .................................................... 28
Figure 4: Scatter plot showing predicted Alkaline Phosphatase and phosphorus levels ........................................................................................................... 33
ABSTRACT

Background
Over the past 20 years, there has been an increase in the survival of infants born prematurely as a result of better newborn care and facilities. Preterms are at increased risk of various problems including metabolic bone disease.

Metabolic bone disease (MBD) of prematurity has been defined as a decrease in the bone mineral content relative to the expected level of mineralization for a fetus or infant of a similar size or gestational age plus biochemical and/or radiographic (1). Preterm neonates are predisposed to MBD of prematurity as they fail to accumulate the essential minerals, namely calcium and phosphorus, required for bone growth that are transferred from the mother to fetus in the last trimester. Children who develop MBD are at risk of poor growth and weight gain, prolonged need for ventilation and as a consequence delayed hospital discharge and risk of rickets of prematurity later on in life. MBD may go undetected as significant demineralization has to occur before it is clinically evident. Screening for MBD is therefore essential early in the course of disease and can be done by assessing bone biochemical markers such as alkaline phosphatase, serum calcium and phosphorus levels.

Study Objective
To determine the prevalence of metabolic bone disease of prematurity, using biochemical markers, at 6 weeks post-natal age at Kenyatta National Hospital.

Methodology
This was a cross sectional study conducted at Kenyatta National Hospital, newborn unit and outpatient department.
Preterm neonates between 28 weeks and 34 weeks gestation and a birth weight between 1,000 g and 2,000 g, feeding on mother’s own milk, were recruited. Anthropometric measurements were taken after birth. The patients were followed up and at 6 weeks of age and their biochemical markers analyzed. These bone biochemical markers included serum Alkaline Phosphatase (ALP), calcium and phosphorus levels. Anthropometric measurements were taken again at 6 weeks post-natal age. Risk factors were retrieved from the patients' files. The risk factors assessed included feeding history, medication history and co- morbid conditions.
Results
Eighty two neonates were screened for MBD at 6 weeks of life with 46.3% being male while 53.7% were female. The mean gestational age and birth weight was 31.5 weeks (SD 1.8) and 1587.3 g (SD 254) respectively. Of the growth parameters at birth, the mean head circumference was 30 cm (SD 1.6) and the mean length was 39.9 cm (SD 2.56). At 6 weeks, the mean weight increment was 15.9g/day (SD 8.9), length was 0.4cm/week (SD 0.2) and head circumference was 0.4cm/week (SD 0.6). The median day for initiation of feeds was day 4 (IQR 3-4) while the median day for reaching full feeds was day 10 (IQR 8-14).

There was no association found between neonatal sepsis, neonatal jaundice, necrotizing enterocolitis, respiratory distress, asphyxia and MBD (p>0.05). Of the neonates screened, 49% were found to have hypophosphatemia while 4.8% had hypocalcemia. The prevalence of MBD was found to be 17% (95% C.I 11-28%).

Conclusions
The prevalence of MBD of preterms was high at 17% in KNH. There was no significant association between feeding practices, medications used and certain co-morbid conditions with MBD.

Recommendations
Screening of preterms gestational age less and equal to 34 weeks and less than 2000g from 6 weeks post-natal age.
1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The survival rate of preterms has increased over the past 20 years resulting in a growing cohort of infants, which has been attributed to establishment of appropriate neonatal intensive care units with enhanced respiratory support facilities. These preterms are at risk of multiple neonatal morbidities such as cerebral impairment, broncho-pulmonary dysplasia, growth failure, retinopathy of prematurity, hearing impairment and including rickets or osteopenia of prematurity now known as metabolic bone disease (MBD).

Metabolic bone disease as Rustico et al. defined it as the decrease in the bone mineral content relative to the expected level of mineralization for a fetus or infant of similar size or gestational age plus biochemical and/or radiographic changes (1). MBD can complicate into rickets and osteopenia. Rickets is defined based on radiographic features in the growing ends of long bones and is historically related to Vitamin D deficiency, while osteopenia implies low bone mineralization (2).

The aetiology of MBD is multi-factorial. Risk factors associated with MBD include prematurity, low birth weight, use of certain medications (e.g.: furosemide, dexamethasone, aminophylline), prolonged periods of total parenteral nutrition, immobilization, delayed establishment of enteral feeds and inadequate postnatal intake of the required vitamins and minerals i.e. vitamin D, calcium and phosphorus. The incidence of MBD is inversely correlated to gestational age and birth weight. Most of the bone mineralization process takes place in the third trimester. The key vitamins and minerals required for this process are calcium, phosphorus and vitamin D. In the last trimester 80% of calcium and phosphorus is transfer (3). Therefore preterms are not able to achieve sufficient levels of these minerals.

The exact prevalence of MBD is not clear due to conflict on screening practices and diagnostic parameters. However, it has been estimated to occur in 16-40% of very low birth weight (<1500 g) and extreme low birth weight (<1000 g) (4,5). Radiographic features of rickets have been found in 55% of extreme low birth weight infants and in 23% of very low birth weight infants (4). The prevalence is higher in breastfeeding infants in contrast to those on preterm formula (21). A study by Siddhartha et al. found the prevalence of radiological rickets (loss of the dense metaphyseal line, increased
submetaphyseal lucency, fraying of metaphyses, with splaying and cupping with or without evidence of fractures) in LBW neonates fed exclusively on breast milk to be 7% and 17% by the end of the 3rd month and 6th month of birth respectively(6). A local study by Oyatsi et al. showed rickets of prematurity to be high at 58.8% at the end of 6 months in LBW neonates at KNH (7).

MBD typically presents at 6 -12 weeks after birth (1). It remains asymptomatic for several weeks before its clinically detected. Radiographic changes may be seen only after 20% demineralization has occurred. Fractures may be detected after severe demineralization (>40%) and may be seen in 10-20% of LBW infants while other clinical features of rickets can also be present (8). Derangements in biochemical markers can be detected, from 2-4 weeks after birth, before radiographic changes and fractures occur. These biochemical markers include hypophosphatemia (<5.6mg/dl or 1.8mmol/l), which can occur as early as 7-14 days after birth. Another marker is elevated alkaline phosphatase (>800 IU) from 6 weeks after birth.

MBD is a complication of prematurity that impairs weight gain as well as affects linear growth. Complications such as rib fractures may make extubation from mechanical ventilation difficult and prolong hospital admission.

Human milk has low levels of calcium, phosphorus and vitamin D. Even 180-200mls/day of feeds of unfortified human milk provides approximately a third of the level of in utero calcium and phosphorus accretion in preterms (9). Infants on full feeds with fortified breast milk reach an optimal level of mineral intake with approximately 180-220mg/kg/day of calcium and 100-130mg/kg/day of phosphorus (10).

Prevention and early treatment of MBD of preterms has been shown to prevent the incidence of rickets of prematurity as well as reduce neonatal fractures, development of rachitic changes and improve linear growth and bone mass in the long term. In the KNH NBU, preterms less than 34 weeks are started on multivitamins from 2 weeks post-natal age or on attaining full feeds, whichever comes first. The multivitamin syrup contains 200 IU/L of vitamin D which is line with the AAP recommendations (10). We currently have no routine timing of screening or supplementation of calcium and
phosphorus. Mothers are encouraged to feed preterms exclusively on breastmilk which remains unfortified.

This study aims to assess the prevalence of MBD in preterm infants currently feeding exclusively on breast milk and on supplements of Vitamin D alone, at 6 weeks of age as well as the risk factors for MBD. This will inform implementation of policies and guidelines on the need for screening for MBD and supplementation of calcium and phosphorus in preterms and VLBW neonates.

1.2 Pathophysiology

Skeletal Development in Utero

The fetal skeleton develops early in the first trimester. The cartilaginous precursors undergo proliferation and differentiation and progressive ossification. By 6 weeks gestation, the primitive skeleton has formed from mesenchymal condensation. The chondrocytes hypertrophy and the perichondral cells differentiate into osteoblasts which form a bone collar around the cartilaginous core. The formation of bone occurs by one of the two processes. In endochondral ossification, the osteoblasts form the primary trabeculae by laying down an osteoid matrix, and bone growth occurs by replacement of the cartilaginous core. These primary sites of ossification, in the axial and appendicular skeleton, appear by the end of the third trimester. Membranous ossification occurs by osteoid being deposited without cartilaginous precursors. This involves the skull, maxilla, and mandible. The cartilage at the end of long bones is organized into growth plates and allows for increase of bone length at about 1.2 cm/wk. Bone volume increases with increasing gestational age, primarily due to increased trabecular thickness. Proliferation of the cartilaginous precursors, progressive ossification, and mineralization determine bone growth in utero. These processes are influenced by the developing vasculature, the availability of nutrients and various hormones (thyroid hormone, growth hormone [GH], pituitary hormone, parathyroid hormone-related peptide [PTHrP]), cytokines, and vitamins (A, C, and D) (11).

Chondrocyte differentiation and proliferation is regulated by PTHrP and transforming growth factor-beta. GH causes chondrocyte proliferation and increases concentrations of insulin-like growth factor (IGF-1) which acts in an autocrine/paracrine fashion to increase chondrogenesis.
Essential minerals in bone formation are calcium and phosphorous. The highest amount of calcium, approximately 99% and 80% of phosphorus are present in the skeleton as microcrystalline apatite \((\text{Ca}_5\text{(PO}_4\text{)}\text{OH})\) at term gestation. Only 1% of the total body calcium is found in the extracellular compartment. 50% of total serum calcium is in the ionized form which is biologically active while 8–10% is bound to organic and inorganic acid and the remainder is protein-bound. The formation of apatite occurs when calcium and phosphorus are available in optimal proportions. 60% of the whole body magnesium is also found in bone matrix. The essential minerals required for bone mineralization are calcium and phosphorus. 80% of calcium and phosphorus transfer occurs in the last trimester. The minerals are 20% higher in the fetus as a result of active transport from the mother to the fetus via the calcium pump on the basement membrane of the placenta (12). The calcium gradient between the mother and the fetus is 1:4 (13). Calcium transport appears to be controlled by Parathyroid Hormone-Related Peptide and Parathyroid Hormone (PTH). The formation of 1,25-dihydroxyvitamin D \([1,25\text{(OH)}_2\text{D}]\), the active form of Vitamin D, also promotes the flow of calcium across the placenta. The manner in which phosphorus is transferred is less clearly understood but is suspected to be through an active transport process mediated partly by 1,25-(OH)2D activated by PTH (14). The accretion of calcium and phosphorus rises exponentially during the third trimester because of the high rate of intrauterine growth. Total body calcium content increases from approximately 5 g at the end of the second trimester to 30 to 35 g by term. The peak accumulation rate of calcium in the third trimester is 120 to 160 mg/kg/d and 60 to 75 mg/kg/d for calcium and phosphorus respectively (3).

**Post-natal bone physiology**

Newborns experience a hypocalcemic phase shortly after birth due to a reduction of maternal oestrogen and a postnatal increase in PTH (1). A physiological nadir of calcium is reached in 24 to 48 hours of birth (15).

Calcium absorption from the intestines occurs passively as well as by active transport which is vitamin D dependent. Due to the immature gut of the pre-term, there’s a net reduction of calcium and phosphorus absorbed. This is compounded by the low calcium and phosphorus levels in preterm breast milk.
The common biochemical abnormalities in MBD include low phosphate and rising alkaline phosphatase levels. Low phosphorus levels manifest as the first indicator of deranged mineral metabolism, occurring from day 7-14 of post-natal life. (16). Phosphate deficiency inhibits PTH production which in turn inhibits urinary phosphate wasting. 1,25(OH)2D synthesis is activated to increase intestinal absorption of calcium and phosphate. Phosphate deficiency affects calcium balance, resulting to hypercalcemia, hypercalciuria, and nephrocalcinosis. In response to low phosphate levels, the kidney increases phosphate reabsorption. Tubular reabsorption of phosphate (TRP) is a reflection of the renal absorption of phosphate. It is a calculated from the ratio of phosphorus and creatinine in serum and urine. Increased TRP (>95%) with hypercalcemia and hypercalciuria can suggest hypophosphatemia.

These changes usually seen together with elevated alkaline phosphatase (ALP). ALP is the sum of bone, liver and intestinal isoforms. The bone isoform contributes to about 90% and representing a marker of bone mineralization (11). A physiologically rise in ALP levels is seen in the first few weeks and plateaus around 5-6 weeks. Rising ALP levels after 6 weeks usually indicates low mineral intake and often seen in MBD.

Bone loading influences bone formation in utero(17). There are 2 types of bone loading. The first is the movement of the fetus against the uterus, more so the extremities. A term infant, with a well developing neuromuscular system, gains the full effect of this form of bone loading, but pre-term infants do not experience this bone loading in the third trimester. The second is the active and passive attachment of muscle. Weight gain increases during the last trimester, with the velocity peaking at 34weeks gestation reaching a plateau from 37weeks. This increase in weight during the third trimester enhances the bone load hence increases the bone formation (18).

Preterm babies tend to be hospitalized for long periods of time with other co-morbidities which renders them inactive. This inactivity and immobilization stimulates bone resorption by osteoclasts. Poor weight gain and muscle growth postnatally also contributes to poor bone loading in preterms.
1.3 Risk Factors for MBD

The aetiology of MBD is multifactorial. The risk factors can be prenatal or postnatal. Prenatal risk factors include conditions causing placental insufficiency such as hypertension, diabetes, anemia, smoking. Post-natal risk factors include prematurity, low birth weight, exposure to certain medications, prolonged immobilization (18), long term parenteral nutrition (19) as well as delayed establishment of full feeds. Poor bone mineralization has been associated with neonatal sepsis, bronchopulmonary dysplasia, cerebral pathology, neuromuscular conditions leading to prolonged immobilization, acidosis, necrotizing enterocolitis (34) and also cholestasis. These are common neonatal conditions.

Prematurity is the greatest risk factor for MBD. Active and passive trans-placental transfer of calcium and phosphorus occurs during the third trimester leading to 80% of these minerals being accreted in the fetus for bone formation (3). Preterm birth also deprives the neonate of bone loading gained through intrauterine movement against the uterine wall especially during the last trimester.

Metabolic bone disease is seen more in very-low-birth-weight infants and extreme-low birth-weight infants. Radiological changes characteristic of rickets have been found in 55% of infants with a birth weight of less than 1000 g and in 23% of infants weighing less than 1500 g at birth. Of infants with a birth weight of less than 1500 g, 24% of them have fractures (4).

Certain drugs such as corticosteroids and methylxanthines used for prolonged periods lead to increased bone resorption with release of calcium and subsequent decrease in the bone mineral content. Prolonged use of loop diuretics increases urinary excretion of calcium. These agents inhibit reabsorption of sodium and chloride at the loop of Henle. Calcium reabsorption is dependent on the active reabsorption of these ions and as a result calcium reabsorption is inhibited. The resulting hypercalciuria increases the risk of nephrocalcinosis.

Immobilization may be due to severe illness, sedation, paralysis or certain congenital conditions (e.g.,: osteogenesis imperfecta, spina bifida). Failure of bone strain in these conditions impairs mechanisms and signals required to transform bone strain into bone
remodeling to increase cortical bone mass (18). Immobilization also promotes bone resorption by osteoclasts and urinary calcium excretion.

Preterms on Total Parenteral Nutrition (TPN) for more than 2 weeks have been seen to be at an increased risk for MBD. This is due to inadequate amounts of minerals including calcium and phosphorus in some TPN solutions so as to ensure its solubility. TPN solutions also, contaminated with aluminum, impair bone calcium uptake (19).

Some nutrients are initially increased in the milk of mothers who deliver prematurely, but the amounts of calcium, phosphorus, protein, and other bone mineral nutrients in unfortified human milk are inadequate to meet the needs of the VLBW infants during growth. Unfortified preterm milk contains 30mg/100kcl and 20mg/100kcl of calcium and phosphorus respectively, which is inadequate for preterm growth (20). A systematic review and metanalysis showed that preterm milk contains 25mg/dl and 10mg/dl of calcium and phosphorus respectively (24). A preterm on 180-200mls/kg/day of unfortified breast milk, the calcium and phosphorus absorbed is about 70% and 80% respectively, and is only a third of that accumulated in utero(9). Although formula milk has higher concentrations of calcium and phosphorus than human milk, its bioavailability is quite different. The absorption of calcium in infants fed with formula milk is less than with human milk, ranging from 35 to 60% of the intake (21).

Delayed enteral feeding or feeding restriction of preterms may be due to their immature physiology and poor absorptive capacity. They are pre-disposed to malabsorption conditions such as necrotizing enterocolitis (34), and clinical states affecting early and adequate nutrition to match intrauterine accretion and growth rates. This pre-dispose them to poor post-natal growth as well as risk of MBD.

1.4 Screening and Diagnosis of MBD of Pre-terms

Screening: Many screening tests are available but there is no uniformity with regard to optimal screening. Choosing whom to screen in the developed world is driven by risk factors. Screening of MBD is usually based primarily on gestational age, with thresholds less than 26 to 36weeks. Other criteria includes birth weight less than 1,000 g, total parenteral nutrition duration of more than 2 weeks, X-ray findings of osteopenia
or fractures, repeated diuretic use, exclusive breast feeding with or without fortification, small for gestational age and intra-uterine growth restriction status (22).

The commonest biochemical abnormalities observed in MBD include hypophosphatemia and hyperphosphatasia. Common screening tests include alkaline phosphatase (ALP) levels, serum phosphate and serum calcium. Others use X-ray findings for diagnosis in response to abnormal screening laboratory tests or alone (22). Other tests include urine calcium and phosphorus, urine calcium: creatinine ratio, parathyroid hormone, serum magnesium, vitamin D. These tests together with tubular reabsorption of phosphate are “additional tests” that have been used to monitor disease progression or to follow up the initial screening (22). Less widely used tests include urinary deoxypyridinoline (Dpd) and Carboxy-terminal telopeptide of type 1 collagen (B-CrossLaps).

Alkaline Phosphatase: There is a physiological increase in alkaline phosphatase (ALP) over the first few weeks of life which plateaus around 5-6 weeks. High ALP levels beyond 6 weeks of life represents low mineral levels and usually goes together with MBD. ALP levels have however not been shown to be consistent with the severity of hypo-mineralization. Very high levels of >1000IU/L of ALP have been shown to be suggestive of rickets. A higher specificity is seen when used together with serum phosphorus levels (23).

Phosphate and Calcium: Hypophosphatemia is the first indicator of deranged mineral metabolism, from 7-14 days postnatal. Low phosphate levels suppresses PTH which prevents urinary phosphate wasting but activates 1,25(OH)2D to increase intestinal absorption of calcium and phosphate. Phosphate deficiency therefore alters calcium balance, which can progress to hypercalcemia, hypercalciuria and nephrocalcinosis.

Extreme premature neonates have a lower threshold for phosphate in urine and increased excretion, even with low serum levels of phosphate. As a result of phosphate deficiency, renal phosphate resorption increases, and hypercalciuria may be observed paradoxically due to inadequate phosphorus forming crystal apatite(18). The detection of about 4mg/kg/day of urinary phosphate and/or calcium (urinary calcium of >1.2mmol/L) is an indication of phosphate deficiency and consequently of MBD.
development. Urine phosphate and calcium may be helpful in assessing bone turnover, are measurable in urine and non-invasive. Phosphate deficiency and may be helpful in detecting bone resorption in pre-terms (1). Phosphorus levels of <5.6mg/dl (1.8mmol/l) yields 70% sensitivity with 100% specificity (25) when used together with ALP levels if >900IU/L.

Tubular phosphate resorption along with calcium excretion may be useful in identifying bone resorption. To calculate the TRP, the ratio of phosphate clearance to the creatinine clearance is used and reflected as a percent. A TRP of more than 95% together with calciuria correlates with phosphate deficiency.

Urinary deoxypyridinoline: Urinary deoxypyridinoline (Dpd) is a specific and sensitive marker of bone matrix resorption but is not widely used. Carboxy-terminal telopeptide of type 1 collagen (B-CrossLaps), measured in serum, is also a marker of bone resorption (26) but also limited in clinical use.

X-rays: Radiologic characteristics of MBD are a late finding. These include reduced cortex, cupping, fraying and widened epiphysis. These radiological changes may not be obvious until approximately 40% decrease of bone mineralization has occurred (33). Fractures may also be seen on x-ray.

Dual energy X-ray absorptiometry (DXA) is technique with high precision and accuracy for measuring BMC and bone mineral density. DXA values are a function of the size, volume and density of the bone. DXA measures a three-dimensional structure in two dimensions, therefore a smaller bone can have a lower radiologic density despite having similar physical density. Therefore, low radiologic density may be suggestive of a small size of otherwise normal bone. The radiation exposure is low (3 mRem), allowing for repeated use. However, it is difficult to show acute changes of bone density, clinical availability is very limited and reference ranges for premature infants are lacking.

Diagnosis: MBD does not manifest clinically silent until severe demineralization has already occurred (~40%). Clinical features of low bone mineralization are skull deformities (sutural diastasis, enlarged fontanelles, craniotabes, frontal bossing),
widened wrists, thickened costochondral junctions (rachitic rosary), and fractures. Bone softening and rib fractures can progress to development of pulmonary changes, respiratory distress, delay in weaning off mechanical ventilators (27).

The diagnosis of MBD is done mainly by the biochemical markers in serum as mentioned above (hypophosphatemia and markedly elevated ALP). It has been suggested that serum alkaline phosphatase levels higher than 900 IU/l together with a serum phosphate level lower than <5.6mg/dl (1.8 mmol/l) has a diagnostic sensitivity of 100% and specificity of 70% (25).

1.5 Treatment and Prevention of MBD
Optimizing nutrition is the main strategy for preventing as well as treating MBD. This involves ensuring adequate quantities of the essential vitamins and minerals, namely, calcium, phosphorus and vitamin D. Other measures include limiting exposure to drugs that reduce calcium and phosphate stores (e.g., loop diuretics, methylxanthines) or increase bone resorption (e.g., glucocorticoids), and enhancing physical activity through physiotherapy.

Early enteral feeds, decreasing the duration of parenteral nutrition, breast milk fortification, and use of specialized preterm formula can decrease the risk of MBD. Use of human milk fortifiers or preterm formulas can be used to increase the amount of calcium and phosphorus levels in feeds. In VLBW infants fed mother's own milk, WHO recommends daily calcium and phosphorus intake of 120 - 140 mg/kg/day and 60 - 90 mg/kg/day respectively and daily vitamin D intake of 400 - 1000 IU/day (28).

According to the AAP, an intake of 150 - 220 mg/kg/day of Calcium and 75 - 140 mg/kg/day of phosphorus is the daily recommended intake (10). The daily Vitamin D supplementation recommendations is 200 - 400 IU/day for preterm infants (10). Physical therapy has been shown to increase weight and length as well as improve short term bone mineralization (37).
1.6 Complications of MBD of Preterms

MBD can lead to both short term and long term morbidity and mortality of preterms. If left untreated, it can progress to rickets of prematurity later in life resulting in short stature and poor extrauterine growth.

The softening of bones due to poor mineralization predisposes these infants to fractures, and rib fractures may worsen respiratory difficulties experienced by the preterms. This further causes increased ventilator dependency with its associated complications such as broncho-pulmonary dysplasia, feeding difficulties and may prolong hospital stay.
2. STUDY JUSTIFICATION AND UTILITY

In the developing world, preterm complications are the leading cause of death among children under 5 years of age.

There is an increasing cohort of preterms and VLBW infants who are surviving due to the establishment of our NBU and more intensive respiratory support facilities. These infants are at risk of several complications including metabolic bone disease, which can later develop into rickets of prematurity if not detected or prevented early.

Preterms are at risk of MBD as they fail to accumulate the essential minerals, namely calcium and phosphorus, for bone health during the third trimester as well as feeding practices. On feeding of the LBW newborn, WHO strongly recommends initiating feeds on the first day of life with mother’s own breast milk for both the LBW and VLBW neonates (28). Breast milk is the best nutrition for preterms as it reduces morbidity, mortality and improves neurodevelopment of infants. However the calcium and phosphorus levels in breast milk are low and inadequate for the growing preterm.

MBD can go undetected for a long time. Old fractures and cortical thinning may be seen on plain radiographs as a result of poor bone mineralization are not usually evident until the BMC has decreased to 40% (29). Failure to identify cases by early screening and delayed treatment of MBD may lead to complications such as development of respiratory complications (27) that may require prolonged mechanical ventilation, fractures, poor growth and weight gain and prolonged hospital stay. Rickets of prematurity develops as a result of failure to detect those at risk, inadequate treatment and prevention of MBD. The prevalence of rickets of prematurity at 6 months in KNH is high at 58.8% (7).

The American Academy of Paediatrics recommends screening practices, prevention and treatment by supplementation of calcium and phosphorus in preterms. This has been shown to reduce the incidence of MBD, improve the rate of linear growth and weight gain as well as reduce hospital stay.

Currently in our new born unit the only preventive measures for rickets of prematurity is the supplementation of vitamin D at 200 IU from 2 weeks post-natal age. Despite
WHO recommendations on calcium and phosphorus supplementation in VLBW neonates, this has yet to be implemented in our facility policies on management and care of the newborn. We lack local statistics regarding the number of preterms affected by MBD since there are no routine screening or supplementation of calcium and/or phosphorus practices.

This study seeks to assess if screening of neonates should be introduced as part of the routine work up of preterms as part of their risk assessment of conditions related to prematurity. It will also guide clinicians on the timing of screening and need for follow up of the biochemical markers in patients with deranged calcium or phosphorus levels and rising alkaline phosphatase.

If this study shows that there’s a high prevalence of MBD, screening of metabolic bone disease of prematurity at 6 weeks of age, using readily available bone biochemical markers such as ALP, serum calcium and phosphorus, these can then be used to assess risk of disease in our NBU from 6 weeks post-natal age in preterms and VLBW neonates. This study will also be able to highlight certain risk factors associated with MBD in our set up and enable healthcare workers focus on these vulnerable groups when screening and treating for MBD. This will also guide our newborn policies of care in our unit on timing of screening and need for supplementation in preterms.
3. STUDY OBJECTIVES

**Question:**
What is the prevalence of Metabolic Bone Disease, using biochemical markers, at 6 weeks postnatal age for preterms of 28 weeks to less than 34 weeks gestation with a birth weight between 1000 g and 2000 g, followed up at the Kenyatta National Hospital?

**Primary Objective**
To determine the prevalence of MBD of prematurity using biochemical markers at 6 weeks post-natal age at KNH.

**Secondary Objective**
To identify the risk factors for MBD of prematurity such as birth weight, feeding practices, certain medications and medical conditions, at Kenyatta National Hospital.
4. RESEARCH METHODOLOGY

4.1 Study Design
Cross-sectional study.

4.2 Study Area
The study was undertaken in the newborn unit and neonatal outpatient clinics, all located at Kenyatta National Hospital (KNH).

The newborn unit is located on the first floor of KNH. It accommodates neonates admitted from labor ward, maternity ward and theatre as well as referring facilities' newborn units.

On admission to the new born unit, neonates are received into the admission room where their vital signs and anthropometric measurements are taken and reviewed by the admitting doctor, usually a paediatric resident. Once the patient has the appropriate investigations performed, treatment commenced, and patient stabilized, the patient is then transferred to a designated room based on their clinical status, medical condition and supportive requirements. Preterms less than 34 weeks and low birth weight babies are started on multivitamins and folate from 2 weeks post-natal age, after attaining full feeds, and iron supplements at 28 days of life.

4.3 Study Population
The study population included infants followed up at Kenyatta National Hospital born between 28 weeks and 34 weeks gestational ages and birth weight between 1000 g - 2000 g.

Case definitions:
Metabolic bone disease is the decrease in the bone mineral content relative to the expected level of mineralization for a fetus or infant of a similar size or gestational age plus biochemical and/or radiographic changes (1). The diagnosis of MBD was made when a patient presented with the following:

- Serum phosphorus levels less than 1.8 mmol/l,
- Alkaline phosphatase more than 900 IU

(Plus, or minus serum calcium (albumin corrected) less than 2.12 mmol/l)
Neonates identified with the above biochemical markers were diagnosed to have metabolic bone disease.

**Inclusion criteria:**
Each of the patients had to fulfil the following criteria to be recruited for the study:
- preterms babies from 28 weeks to 34 weeks gestational age by date
- birth weight between 1000 g and 2000 g
- feeding on own mothers breastmilk
- informed written consent by parent/guardian

**Exclusion criteria:**
Patients meeting the exclusion below were not included in the study:
- Infants feeding exclusively on formula

**4.4 Sample Size Calculation**
The sample size was determined using the Cochran’s formula sample size in prevalence studies. The formula was used to determine the number of subjects required to estimate the prevalence of MBD in neonates at six weeks with a margin of error of ± x%:

\[
 n = \frac{Z_{\alpha/2}^2 \times P(1-P)}{d^2}
\]

Where:

- \(Z_{\alpha/2}\) = 1.96 representing 95% level of confidence
- \(Z_{\alpha/2}^2\)
- \(P = \) is the proportion preterm newborn estimated to develop MBD, a previous study by Dr. Oyatsi in KNH (7).(58.8%)
- \(1-P = \) 1 minus the proportion preterm newborn estimated to develop MBD previous study(7). (1-0.588)
- \(d = \) margin of error (set at x%)

\[
 n = \frac{1.96^2 \times 0.588(1-0.588)}{0.1^2}
\]

16
n = 94; with a markup of 10% to allow for lost to follow up and mortalities, the effective sample size calculated for the study was 104

4.5 Sampling Methods
Consecutive sampling was used to recruit preterm newborns in KNH during the study period. Every newborn less than 24 hours of age meeting the study inclusion criteria was recruited until the required sample size was achieved.

4.6 Study Variables
The outcome variables include:
Serum Phosphorus of less than 1.8 mmol/l
Alkaline Phosphatase of more than 900 IU/L
Plus, or minus Calcium levels of less than 2.12 mmol/l

4.7 Study Tools
At the point of recruitment, the patient’s details such as gestational age and birth weight were recorded. The patient was allocated a serial number (see appendix 2, section A). The serial number corresponds to the patient’s hospital number and was kept among confidential records only known to the principal investigator.

Laboratory results which include serum levels of corrected calcium, phosphorus and ALP, were reported and recorded after analysis (see appendix 2, section C).

4.8 Study Period
December 2017 to April 2018, at which point the expected sample size was reached.

4.9 Selection and Enrolment of Participants and Consent Procedure
The potential participants were identified sequentially, based on gestational age or birth weight, at admission into the KNH NBU.

The patients were identified based on gestational age of 28 to 34 weeks and birth weight between 1000 g to 2000 g. Gestational age was ascertained using the Ballard method within 7 days of birth. Patients were weighed using the infant weighing scale at admission to the newborn unit.
The patients identified to be eligible for the study had the purpose and methods of the study explained to the parent of the specific patients, allowing them to provide voluntary informed consent. The purpose of the study, the study procedures to be used and potential benefits and risks of participating in the study were discussed with the parent following a pre-designed format. Any pertinent questions from the parents, regarding the study, were answered prior to signing of the consent form. A parent accepting to take part in the study was then given a consent form to sign, which was countersigned by the investigator. A copy of the consent form was given to the parent who consented to the study and a copy retained by the investigator.

Participants discharged during the study period were followed up in the new born unit and neonatal outpatient clinic. Patients discharged before the end of the study period had clinic appointment dates set which corresponded to the timing of screening. Parents with access to mobile phones were reminded of appointment dates by text or phone call.

4.10 Study Procedures
4.10.1 Clinical Procedures
Patients were weighed nude with the use of a calibrated infant scale, EBSA-20, to the nearest 5 g. Weight was taken at birth and at 6 weeks. The head circumference at birth and at 6 week post-natal age were measured at the largest occipito-frontal circumference by means of a non-stretchable paper tape to the next succeeding millimeter. The patient was also assessed for intrauterine growth using the Lubchenco intrauterine growth curves and grouped as either small, appropriate or large for gestational age.

All subjects who have fulfilled the inclusion criteria received an oral multivitamin supplement starting at 2 weeks that contains 200IU/L of Vitamin D in 5mls (as per the standard practice).

Patients' screened and noted to have deranged biochemical markers pointing to MBD had supplementation of calcium and phosphate prescribed and follow up recommended.
4.10.2 Laboratory Procedures

Sample Collection and Transport: The biochemical markers, ALP, serum phosphorus and calcium, were measured at 6 weeks postnatal age. Trained medical personnel drew the blood samples. A venous blood sample of 2 mls was drawn using an aseptic technique. Aseptic blood withdrawal was ensured by washing of hands with soap prior to wearing of clean gloves. Alcohol swabs were used to clean the site of needle puncture at least twice before blood withdrawal. Once drawn, the blood was placed in a yellow capped vacutainer blood collection tubes with anti-coagulant that were provided by Lancet Laboratory. Each vacutainer was clearly labelled with the patient’s serial number. It was then stored at room temperature in a sealed box and transported by a motor-vehicle to Lancet Laboratory within the same day. Samples were analyzed on the same day as collection. The cost of the tests carried out were covered by the principle investigator since the test is not part of the standard practice.

Methods: Quality control was performed by an ISO15189 accredited laboratory to ensure standardization of results. Tests were compared for accuracy and precision and each sample compared to the controls to ensure validation of the measurements.

Internal quality control was done on a daily basis, one in the morning and one in the evening after all tests have been completed. Internal quality control was done by running a known quality control sample along with the tests to confirm the validity of the values of the tests. An external quality control was done on a monthly basis.

COBAS INTEGRA 400/700/800 was used to analyze ALP and Calcium while COBAS INTEGRA 400/800 was used to analyze Phosphorus. Colorimetric assay was in accordance with a standardized method. COBAS INTERGRA analyzers automatically calculate the analyte activity of each sample.

These results were recorded and made available to the principle investigator on soft copy within 48hours of sample collection for recording in the data collection sheet (see appendix 2, section C).

4.10.3 Training of research assistants.

Two research assistants, who are clinical officers, were involved in the project.
The research assistants were trained for a duration of a week, before the study, on drawing the blood samples using an aseptic technique, use of the Ballard scoring tool, weight taking and measuring of head circumference and recording of data.
5. DATA COLLECTION, MANAGEMENT AND ANALYSIS

5.1 Data Collection
The laboratory findings were documented in the data collection form (see appendix 2, section B) on a weekly basis.

5.2 Data Management and Analysis
The patients' demographics were recorded on admission (see appendix 2, section A) and random numbers assigned. Information on the data collection form (see appendix 2, section A) were collected from the file during admission. Patients discharged before the end of the study were reviewed and screened, in the neonatal outpatient clinic, at 6 weeks post-natal age and additional data obtained from the files.

A separate log book was kept with the principle investigator which included the full patient’s details as well as parents contacts and stored in a locked file cabinet. The patients' details were copied in a password-protected database.

The data collection, entry and cleaning were based on Standard Operating Procedures (SOPs) prepared prior to commencement of the study. All study personnel (data collection and entry, laboratory procedures) were trained on the study aims and methodology.

Data entry, cleaning and analysis were conducted using Stata Software version 14. Databases were customized using the study tools and included range and validity checks to reduce errors.

Univariate analysis was carried out to describe sample characteristics and determine the prevalence of MBD. Means with standard deviation for normally distributed independent variables and medians with the interquartile ranges for skewed data.

Bivariate analysis using student’s t test was carried out for continuous variables such as gestational age, with outcome variables. Statistical significance was assumed at p < 0.05.
Chi square and Fischer’s test was used to analyze for associations between outcome variables and independent variables. For skewed data, Mann-Whitney U test was used. A scatter plot was generated to determine the correlation between Phosphorus and Alkaline phosphatase. The primary outcome was the percentage of newborns with MBD at 6 weeks after birth.

5.3 Study Limitations
The following limitations were encountered during the study:

1. Loss to follow up after discharge. Some mothers did not have access to mobile phones while others opted to continue clinic follow up at facilities closer to them rather than come to KNH.
2. Untraceable file records. Some files had been misfiled and were untraceable. Clinical data was therefore missing on some patients.
3. Invalid laboratory results that may have been due to collection of an arterial sample rather than a venous sample or a cannulated vessel, altered the biochemical markers.
4. Many mothers may have commenced calcium supplements and exposed their children to sunlight after discharge. This was not evaluated in the questionnaire. This would affect the prevalence of MBD as these factors alter bone metabolism.

5.4 Study Closure and Procedure
Study closure was initiated once the study was completed including data collection, verification and analysis. A closure report in addition to a closure application form shall be submitted to the KNH-UoN ERC for verification and approval. Data on the percentage of newborns found to have MBD and identified risk factors has been recorded and stored until the final study is published. The final database, on which data analysis and publication is based, shall be properly labeled ready for archiving.
6. ETHICAL CONSIDERATIONS

Permission was sought from the Kenyatta Hospital Ethics Research Committee (KHERSC) to collect and analyze data collected in the study as part of the thesis dissertation. Copies of this protocol, the informed consent form as well as any subsequent modifications to either document were presented to the above named committee for written approval prior to commencing the study.

Modifications to the study protocol that affect the patient’s volition to take part in the study, the intent of the study or patient safety was submitted to the KHERSC for written approval prior to incorporating these changes in the study procedure.

The purpose of the study was carefully explained to the patients’ parents with a view to obtaining written consent prior to enrolling any infant in the study. Consent obtained was voluntary and free from coercion.

The laboratory investigations, that have been used as screening tools in developed countries, were employed in this study. The benefits that participants accrued from the study included early recognition of MBD and institution of treatment and follow up recommended. The risks of the study included discomfort during sample withdrawal which was explained to the parent. Laboratory investigation costs were covered by the principle investigator.

Strict confidentiality was observed throughout the entire study period, held in trust by participating investigators, research staff and the study institutions. The study participants were given study identification numbers. Any data consisting of patient identification details were only accessible to the principle investigator. No information concerning the individual study findings was released to any unauthorized third party without prior written approval of the study institution or the Ethics Research Committee.

The overall study findings will be availed to the specialists and staff in the KNH NBU and paediatric out-patient clinic to facilitate appropriate care of the pre-terms at risk.
The study findings will also be presented to the University of Nairobi (UON) Department of Paediatrics and Child Health academic staff and students in fulfilment of the requirements of the M.Med Program.
7. RESULTS

The results include a brief summary of the patient recruitment and sociodemographic characteristics. This is followed by the results on prevalence of disease, the growth and nutritional characteristics, risk factors and finally the results of the biochemical markers of MBD.

A total of 104 newborns were recruited at the NBU and consent given. Only 92 newborns were followed up in the unit or at the neonatal out patient clinic. Of the 104 newborns recruited, 12 newborns were lost to follow up while 10 had invalid results. A total of 82 newborns were screened. The study was carried out between December 2017 and April 2018.

In figure 1 below, the patient recruitment and follow up flow is illustrated.

**Figure 1: Patient recruitment and follow up flow chart**

Newborns with birth weight of 1000g - 2000g, GA 28-34 weeks \( n=104 \)

- Followed up to 6 weeks \( = 92 \)
- Lost to follow up \( = 12 \)
- Invalid lab results \( = 10 \)
- Valid results \( = 82 \)
7.1 Sociodemographic Characteristics

The gender distribution of the sample population is presented in figure 2 below.

Figure 2: Gender distribution

Of the 104 patients who were recruited, only 82 patients were screened at 6 weeks as 12 patients were either lost to follow up or had invalid lab results (insufficient sample, arterial sample, diluted by intravenous fluids) and were hence excluded from the analysis. The characteristics of all 82 neonates who were screened and their anthropometric parameters at birth are presented in Table 1. The Lubchenco intrauterine growth curves were used to classify patients as SGA (< 10th percentile), AGA (10th to 90th percentile) and LGA (> 90th percentile) (38).
A total of 82 patients were screened at 6 weeks with 38 (46.3%) of the neonates being male while 44 (53.7%) were female. The mean gestational age was 31.5 weeks (SD 1.88). The mean birth weight was 1587.3 g (SD 254), mean head circumference of 30 cm (SD 1.6) and mean length at birth of 39.9 cm (SD 2.56). Small for gestational age patients accounted for 12.2% of the patients screened, 69.5% were appropriate for gestational age and 18.3% were large for gestational age.

### 7.2 Disease Prevalence

The prevalence of MBD of preterms from the study is presented in Figure 3.
Of the patients screened, 14 out of 82 patients (17%) had MBD. The 95% confidence interval of patients with MBD was 11-28%.

Table 2: Frequency of MBD between the different age groups

<table>
<thead>
<tr>
<th>Gestational Age</th>
<th>No MBD</th>
<th>With MBD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 - 30 weeks</td>
<td>21</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>30 - 32 weeks</td>
<td>25</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>32 - 34 weeks</td>
<td>22</td>
<td>4</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>14</td>
<td>82</td>
</tr>
</tbody>
</table>

Patients between 28 and 34 weeks were screened for MBD. The patients were divided into different age groups to assess if the frequency of MBD was different at different gestational age groups. The Table 2 above shows the prevalence of MBD between the different age groups. As illustrated using Bonferroni test (p = 1.0), there was no significance in gestational age and prevalence of MBD.
Table 3: Frequency of MBD in relation to birth weight

<table>
<thead>
<tr>
<th>Birth Weight</th>
<th>No MBD</th>
<th>With MBD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1000g</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1000 - 1500 g</td>
<td>19</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>1500g - 2000 g</td>
<td>49</td>
<td>7</td>
<td>56</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>68</strong></td>
<td><strong>14</strong></td>
<td><strong>82</strong></td>
</tr>
</tbody>
</table>

Table 3 compares birth weight and the development of MBD. The patients were grouped based on birth weight i.e those less than 1000g, 1000-1500g, 1500-2000g. The patients in each weight range were grouped into those with MBD and those without MBD. Only 1 patient with a weight of 1000g had MBD, 6 patients with MBD were between 1000g and 1500g, while 7 patients were between 1500g and 2000g. Fisher’s exact test was used to determine if MBD was related to birthweight. In this study it was seen that there was no significant difference in the birth weight and those who developed MBD (p = 0.058)

Table 4: Distribution of MBD based on Intrauterine Growth Assessment

<table>
<thead>
<tr>
<th></th>
<th>MBD (%)</th>
<th>95% C.I</th>
<th>No MBD (%)</th>
<th>96% C.I</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGA</td>
<td>7.1</td>
<td>0.8 - 39</td>
<td>20.5</td>
<td>12.4 - 32.1</td>
</tr>
<tr>
<td>AGA</td>
<td>78.5</td>
<td>48.8 - 93.3</td>
<td>67.6</td>
<td>55.4 - 77.8</td>
</tr>
<tr>
<td>SGA</td>
<td>14.2</td>
<td>33.2 - 44.6</td>
<td>11.7</td>
<td>5.9 - 22</td>
</tr>
</tbody>
</table>

The table above shows the different intrauterine growth patterns and risk of developing metabolic bone disease of preterms. Of the patients who had MBD, 7.1% were LGA, 78.5% were AGA and 14.2% were SGA. Using Fishers exact test, there was no significance found between the intrauterine growth and MBD (p = 0.64).
7.3 Growth Parameters

Table 5: Growth Parameters at 6 weeks

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight at 6 weeks (grams)</td>
<td>2051</td>
<td>223</td>
</tr>
<tr>
<td>Weight increment g/day</td>
<td>15.9</td>
<td>8.9</td>
</tr>
<tr>
<td>Head circumference cm/wk</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Length at 6wks cm/wk</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Regaining birth weight (days)</td>
<td>14.9</td>
<td>8.8</td>
</tr>
</tbody>
</table>

Table 5 shows the univariate analysis of the growth parameters at 6 weeks. Growth velocity was determined by calculating the weight gain per day after the neonate had regained birth weight. The mean weight at 6 weeks was 2051 g (SD 223), while the increment was 15.9 g/day (SD 8.9) and the increment in head circumference was 0.4 cm/week (SD 0.6). The mean day of regaining birth weight after birth was 14.9 days (SD 8.8). The growth velocity was calculated from the time of birth and at 6 weeks.

Table 6: Growth increments in neonates with and without MBD

<table>
<thead>
<tr>
<th>Growth at 6 weeks</th>
<th>With MBD Mean (SD)</th>
<th>No MBD Mean (SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g/d)</td>
<td>16.3 (4.90)</td>
<td>15.9 (9.54)</td>
<td>0.86</td>
</tr>
<tr>
<td>Head circumference (cm/wk)</td>
<td>0.43 (0.24)</td>
<td>0.44 (0.61)</td>
<td>0.98</td>
</tr>
<tr>
<td>Length (cm/wk)</td>
<td>0.45 (0.14)</td>
<td>0.38 (0.25)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

The growth velocity in patients with MBD was 16.3 g/d (SD 4.9) and 15.9 g/d (SD 9.54) in those with no MBD. The increment of head circumference at 6 weeks post natal age was 0.43cm/wk (SD 0.24) in patients with MBD and 0.44cm/wk (SD 0.61) in patients without MBD. The length increment was 0.45cm/wk (SD 0.14) and 0.38cm/wk.
(SD 0.25) in patients with and those who did not have MBD respectively. Using the standard t test, the increments in anthropometric measurements was similar in both neonates with MBD and those without MBD as illustrated in Table 6 above.

### 7.4 Feeding Patterns

The feeding patterns of the neonates was assessed by documenting when enteral feeds were commenced and when full feeds (200mls/kg/day) were reached. The earliest enteral feeds were started on day 2 of life, while the latest was at day 14 of life. The shortest time to reach full feeds was day 7 of life while the longest duration was day 19 of life. The median day (interquartile range (IQR)) for initiation of feeds was day 4 (3-4) of life, while that for reaching full feeds was day 10 (8-14) of life.

In Table 7, the mean day of the feeding patterns and the risk of developing MBD using the standard t test are presented. The mean day of initiation of starting feeds was at day 3.4 (SD 2.49) for patients with no MBD and day 4.3 (SD 3.2) in patients with MBD with a p value=0.28. The mean day of reaching full feeds was at day 10 (SD 6.6) for patients with no MBD and day 11 (SD 3.9) in patients with MBD with a p value=0.87. The p value of both feeding patterns is >0.05 illustrating both timing of initiation of feeds and that to reaching of full feeds were therefore not significant to developing MBD according to this study.

<table>
<thead>
<tr>
<th></th>
<th>Mean (No MBD) (95% CI)</th>
<th>SD</th>
<th>Mean (With MBD) (95% CI)</th>
<th>SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiation of feeds</td>
<td>3.4 (2.7-4.1)</td>
<td>2.49</td>
<td>4.3 (2.2-6.8)</td>
<td>3.2</td>
<td>0.28</td>
</tr>
<tr>
<td>Full feeds</td>
<td>10 (8.9-12.5)</td>
<td>6.6</td>
<td>11 (8,5-13)</td>
<td>3.9</td>
<td>0.87</td>
</tr>
</tbody>
</table>

### 7.5 Risk Factors

Table 8 shows a bivariate analysis between birth weight and gestational age. Of patients with MBD, the mean birth weight was 1566 g (SD 362) while the mean gestational age was 31 weeks (SD 2). Of patients who did not have MBD, the mean
birth weight was 1591 g (SD 229) while the mean gestational age was 31 weeks (SD 2). A standard t test was used to see the relation of birth weight and gestational age to MBD. Both birth weight and gestational age were not statistically significant (p>0.05).

<table>
<thead>
<tr>
<th>Table 8: Bivariate Analysis of risk factors vs presence of MBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Weight (grams)</td>
</tr>
<tr>
<td>No MBD</td>
</tr>
<tr>
<td>N: 58, Mean: 1591, Standard deviation: 229</td>
</tr>
<tr>
<td>p value: 0.74</td>
</tr>
<tr>
<td>With MBD</td>
</tr>
<tr>
<td>N: 15, Mean: 1566, Standard deviation: 362</td>
</tr>
<tr>
<td>No MBD</td>
</tr>
<tr>
<td>N: 58, Mean: 31, Standard deviation: 2</td>
</tr>
<tr>
<td>p value: 0.69</td>
</tr>
<tr>
<td>With MBD</td>
</tr>
<tr>
<td>N: 15, Mean: 31, Standard deviation: 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 9: Proportion of MBD in neonates with the risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBD</td>
</tr>
<tr>
<td>NNJ</td>
</tr>
<tr>
<td>NNS</td>
</tr>
<tr>
<td>NEC</td>
</tr>
<tr>
<td>RDS</td>
</tr>
<tr>
<td>Asphyxia</td>
</tr>
<tr>
<td>PPN</td>
</tr>
</tbody>
</table>

*Fisher’s exact*

The table above shows the proportion of patients suffering from the medical conditions studied to assess if any of them was a risk of MBD. When Fisher’s test was carried out,
all risk factors were not associated with the development of MBD as all the p values were > 0.05

7.6 Biochemical Parameters

Figure 4: Scatter plot showing predicted Alkaline Phosphatase and phosphorus levels

Figure 4 is a scatter plot showing predicted ALP levels and Phosphorus. This demonstrates the weak correlation between levels of inorganic phosphate and Alkaline phosphatase. Correlation coefficient of -0.0809.

Table 10: Calcium and Phosphorus levels in the sample population

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Low</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus</td>
<td>51% (40-62)</td>
<td>49% (37.9-59.7)</td>
<td>0.00</td>
</tr>
<tr>
<td>Calcium</td>
<td>95% (87-98)</td>
<td>4.8% (1.8-12.5%)</td>
<td>0.46</td>
</tr>
</tbody>
</table>
Table 10 presents the proportion of patients with normal or low phosphorus and calcium levels. Normal phosphorus levels were found in 51% of neonates while 49% had hypophosphatemia. Normal calcium levels were found in 95% while 4.8% had hypocalcemia.

Table 11 presents the calcium levels between patients with MBD and those with no MBD. All 14 patients with MBD had normal calcium levels while of those with no MBD, 64 patients had normal calcium levels and 4 had hypocalcemia. There was no significant difference in calcium levels in patients with MBD and those with no MBD (p=0.46).
8. DISCUSSION

In this cross sectional study, the prevalence of MBD of preterms was found to be 17% (95% CI 11-28%) in neonates with gestational ages between 28 and 34 weeks and birth weight of 1000 - 2000g at the Kenyatta National Hospital. The prevalence of MBD in this study is comparable to previous studies. A study done in developing countries showed the prevalence of MBD of preterms in very low birth weight and low birth weight infants to be between 16 - 40% (4,5). Backstrom et al also described the prevalence of radiological rickets to be 23% in VLBW infants (4).

This wide confidence interval may be attributed to the small sample size due to patients lost to follow up and invalidity of some samples. The low prevalence of MBD may be attributed to the supplementation of 200 I.U of vitamin D in the oral multivitamin used as routine standard care in our NBU from 2 weeks of age. Supplementation of Vitamin D is in line with the recommended dose of Vitamin D of 200-400 I.U per day as per the American Academy of Paediatrics guidelines (10). It may also be related to sun exposure of the newborn after discharge, which was not evaluated in this study. The wide range of birth weight, of 1000 g - 2000 g, of the sample population may have also affected the prevalence rate.

The study population comprised of neonates between 28 and 34 weeks and birth weight of less than 2000 g. To assess if MBD was inversely related to gestational age, the sample population was divided into different gestational ages, i.e 28-30 weeks, 30-32 weeks and those more than 32 weeks. The prevalence of MBD was also assessed in different birth weight ranges, i.e less than 1000 g, 1000 g -1500 g, and 1500g - 2000 g. In this study, MBD was not seen to be inversely related to the gestational age or birth weight. This is not the case in similar studies done that illustrated that the risk of MBD increases with decreasing birth weight and gestational age (23,30). An Egyptian study comparing birth weight <1500 g, and gestational age, <34 weeks, showed a significant inverse correlation to both serum ALP levels and radiological evidence of osteopenia in their preterm infants (P<0.005) when evaluated from 8 weeks post natal age (31). This study may have failed to show this relationship as the sample population had a wide range of weight at birth (1000 g - 2000 g).
The distribution of MBD among patients of different intrauterine growth rates (SGA, AGA and LGA) showed no significant difference. This is similar to a study done by Stephen et al. illustrated similar bone mineral content in preterms with a birth weight of 900 g - 2200 g and at 10 weeks post natal age (39).

In preterms, as with term neonates, there is an initial weight loss before accelerated growth occurs. In preterms, their initial growth is faster than term neonates as a means of catch up growth. In the study population, the mean growth velocity as g/day was noted to be 15.9 g/day (SD 8.9). In utero, the foetal growth velocity is 16g/kg/day from 23 to 37 weeks gestation (32). This forms the basis for the current American Academy of Pediatrics (AAP) recommended target growth rate of 15g/kg/day for the ELBW preterm (20). The growth parameters in the neonates with MBD and those without MBD did not differ. According to this study, MBD did not affect the growth velocity in the preterms studied in the first 6 week of life.

The neonates screened were fed exclusively on unfortified mother’s own breast milk. The mean day of life for initiation of feeds was 3.4 days (SD 2.49) in neonates who did not develop MBD as compared to 4.3 days (SD 3.2) in neonates who developed MBD. The timing of initiation of feeds in association to MBD was not significant ( p value 0.28). This is different from studies that have associated delayed feeding practices to development of MBD as well as poor post natal catch up growth, risk of neonatal sepsis and necrotising enterocolitis, which further reduce nutrient absorption and increase risk of MBD.

Neonatal sepsis, neonatal jaundice, birth asphyxia, respiratory distress and necrotizing enterocolitis were the morbidities studied as risk factors of MBD in this study. There was no statistical significance between these risk factors and MBD of preterms. Compared with these results, Eliakim et al (33) reported that neonatal sepsis in premature infants was not associated with biochemical evidence of diminished bone turnover. A similar study comparing the incidence of MBD and sepsis using markers such as C reactive protein and total leucocyte count, showed no significant relation between the incidence of MBD in preterm infants and sepsis as well as NEC (31). This is unlike a finding by Cakir et al who found that necrotizing enterocolititis increases the bone resorption in premature infants which may be related to reduced glucagon like
peptide-2 levels, a new intestinal hormone that is primarily secreted from the distal small intestine (34).

The calcium levels in the patients with MBD and those with no MBD showed no significant difference and were largely maintained within normal parameters. This is in agreement with a study done that reported normal calcium level in osteopenic infants which, as explained, was maintained by parathyroid hormone effect that stimulates calcium reabsorption (35). This was also reported in a study in Egypt that compared calcium levels in osteopenic and non osteopenic neonates of VLBW(31) that showed no difference in calcium levels in both groups.

Hypophosphatemia was noted in 49% of the study population and was seen to have a high association with patients with high ALP. A similar study showed that high plasma alkaline phosphatase was related to bone mineral substrate deficiency with low plasma phosphorus concentrations (P<0.0001) (36).
9. CONCLUSIONS AND RECOMMENDATIONS

9.1 Conclusions

1. The findings from this study indicate that the prevalence of metabolic bone disease of preterms is 17% at KNH which is similar to studies done in developed countries.
2. Growth rates remain unaffected at 6 weeks post natal age for those with and those without MBD.
3. There was no significant association between initiation of feeds, full feeds, neonatal sepsis, neonatal jaundice, respiratory distress, asphyxia and the development of MBD.

9.2 Recommendations

Screening of preterms for MBD should be carried from 6 weeks and serial measurements done up to 12 weeks post natal age. For those found to have MBD, treatment with phosphorus and calcium should be started. Additional studies demonstrating optimal duration of supplementation and treatment should be carried out. For neonates with deranged biochemical markers, such as rising ALP levels, not qualifying for diagnosis of MBD, serial measurements of the biochemical markers should be carried out.
10. REFERENCES


Appendix 1: Consent Information Form

Study Title: PREVALENCE OF METABOLIC BONE DISEASE OF PREMATURITY USING BIOCHEMICAL MARKERS AT 6 WEEKS POST NATAL AGE AT KENYATTA NATIONAL HOSPITAL.

Serial number: ………………………………

Principal Investigator: Dr. Wambui Wanyoike (MB ChB), Paediatric Resident,
University of Nairobi. Telephone Number: +254 719 368 567

Supervisors: Professor R. N. Musoke, Dr Rashmi Kumar, Dr. Lucy Mungai

This Informed Consent form has two parts:
• Consent Information Form - To share information about the research with you
• Certificate of Consent - For signatures if you agree to take part in the research

You will be given a copy of the full Informed Consent Form

I. CONSENT INFORMATION FORM

Investigator’s Statement:

We are requesting you and your child to kindly participate in this research study. The purpose of this consent form is to provide you with the information you will need to help you decide whether to participate in the study. This process is called ‘Informed Consent’. Once you understand and agree for your child to be in the study, I will request you to sign your name on this form. Refusal to participate in the research will not affect the services your child is entitled to in this health facility. Please read this consent information carefully and ask any questions or seek clarification on any matter concerning the study with which you are uncertain.

Introduction:

Metabolic Bone Disease (MBD) of prematurity is a condition affecting children born before 36 weeks gestation or a birth weight of less than 1,500 g. It is caused by lack of the essential minerals required for normal bone growth, namely calcium and phosphorus. These minerals are transferred from the mother to the unborn baby during the third trimester. Babies born before the third trimester fail to accumulate adequate stores of these minerals. The babies have low stores of this minerals which in turn alter normal growth. These babies are at risk of prolonged hospital stay as a result of prolonged need for breathing support from ventilator machines, fractures of their fragile bones and poor weight gain. The babies are also at risk of developing fragile bones known as rickets of
prematurity and shorter height later in life. Bone abnormalities such as rickets or fractures are seen late in the disease and hence the need for early screening, detection and adequate prevention and treatment.

Purpose of the study:
This study seeks to determine the number of preterms and very low birth weight infants who are at risk of developing metabolic bone disease by the sixth week after birth.

Study Procedures:
The babies will be identified on admission to the new born unit based on gestational age of between 28 weeks and less than 34 weeks and birth weight of between 1,000 g and 2,000 g. Weight and head circumference at birth will be measured and recorded. At 2 weeks of age, vitamin D supplement will be given to your baby as per our standard practice. A blood sample will be withdrawn at 6 weeks after birth and sent to the lab for analysis. Weight and head circumference at 6 weeks post-natal age will also be measured and recorded.

We will request for a telephone number where we can contact you for the follow-up appointment and tests if you are discharged from the hospital before the end of the study. Follow up will be conducted in the neonatal outpatient clinic, child welfare clinic or paediatric wards.

Specimens:
A blood sample will be collected at 6 weeks after birth. The amount to be collected will be 2 mls of blood and put in a collecting tube to be analyzed.

Follow up:
The study will require us to test your baby at 6 weeks of life. Any babies recruited from the new born unit and discharged before the end of the study will be followed up at the Neonatal Outpatient Clinic or Child welfare clinic. Reminders of appointment dates via telephone calls will be two days prior. During the visit at 6 weeks, the sample will be withdrawn for the study.

Benefits:
The results of the study will be shared with you and your doctor.
If your baby is discovered to be at risk of MBD, early supplementation of calcium and phosphorus will be recommended as well as adequate follow up.
The results of the research will also be used by other medical practitioners as a guide on when to screen other preterm babies and identify babies early who require any treatment or supplementation.
Risks:
Laboratory investigations involve taking blood samples of 1 ml collected from your baby. All precautions will be taken to ensure it’s an aseptic procedure and to prevent unnecessary bleeding. Procedures will be undertaken to the study participants and will be done with care, with minimal acute or long-term risks to the participants. Samples will be collected by skilled medical personnel.
Refusal to participate will in no way jeopardize the treatment of your child in any way.
Confidentiality:
The information obtained about you, your child and your family will be kept in strict confidence. No specific information regarding you, your child or your family will be released to any person without your written permission. We will use a code number to identify your child in a password protected database and will keep all our paper records in a locked file cabinet. We will, however, discuss general overall findings regarding all children assessed but nothing specific will be discussed regarding your child. We will also, not reveal the identity of you or your child in these discussions.
Voluntariness:
Your decision to have your child participate in this research is voluntary. There will be no financial rewards to you for participating in the study. One is free to participate or withdraw from the study at any point. Refusal to participate will not compromise your child’s care in any way.
II. CERTIFICATE OF CONSENT FORM

Serial Number: ..............................................
Date: ..........................................................

Participant’s Statement:
I ____________________________ am having received adequate information regarding the study research, risks, benefits hereby AGREE / DISAGREE (Circle as appropriate) to participate in the study with my child. I understand that our participation is fully voluntary and that I am free to withdraw at any time. I have been given adequate opportunity to ask questions and seek clarification on the study and these have been addressed satisfactorily.

Parent/Guardian Signature/Thumb stamp:_____________ Date: __________
Parent/Guardian Name: _____________________________

Researcher’s statement:
I ____________________________ declare that I have adequately explained to the above participant, the study procedure, risks, benefits and given him/her time to ask questions and seek clarification regarding the study. I have answered all the questions raised to the best of my ability.

Printed name: ______________________
Signature: ____________________________

For any questions or clarification, please do not hesitate to contact:

Dr. Wambui Wanyoike (Principal Investigator) on +254 719 368 567

Professor Musoke (Supervisor) on +254 721 307 160

Kenyatta National Hospital, University of Nairobi Ethics and Research Committee (KNH-UON ERC), P.O BOX 19676-00202 or Tel. no 2726300-9 Ext. 44355, email uonknh_erc@uonbi.ac.ke

Date____________________
FOMU YA RIDHAA
Kichwa cha utafiti:  KUAMUA KIWANGO CHA MAAMBUKIZI YA UGONJWA YA METABOLIC BONE DISEASE KWA WATOTO WALIOZALIWA KABLA YA WIKI THELATHINI NA NNE AU CHINI YA KILO 1,500 G KATIKA KITUO CHA KENYATTA NATIONAL HOSPITAL.

Mtafiti: Dr. Wambui Wanyoike, Kituo cha Watoto, Chuo kikuu cha Nairobi. Nambari ya dharura pigia +254 719 368 567
Wasimamizi: Prof. R.Musoke, Dr Rashmi Kumar, Dr. Lucy Mungai., Kituo cha watoto, Chuo kikuu cha Nairobi.

Hii fomu ya idhini ina sehemu mbili:
• Sehemu ya Maelezo - kukuelezea zaidi kuhusu utafiti
• Shahada ya Idhini - Sahihi ikiwa umekubali kujihusisha na utafiti huu

SEHEMU YA KWANZA:
Tamko la mtafiti:

Utangulizi:
Ugonjwa wa metabolic bone disease (MBD) ni ugonjwa unayoudhuru watoto waliozaliwa kabla kufikisha miezi thelathini na sita na kilo chini ya 2,000 gm. Ni ugonjwa unayodhuru hali ya mifupa kwa vile madini zinazohitajika, kama vile, calcium na phosphorus, zinazopatikana kutoka mama wakati wa wiki za mwisho wa mimba, hazipatikani kwa hawa watoto. Upungufu wa hizi madini huweza kufanya mifupa ya mtoto kupata madhara kwa urahisi and kuzuia mtoto kuongeza kilo kama watoto wengine na pia urefu. Ugonjwa huu huchukua muda tabia inaweza kutambuliwa, na kwa hivyo vipimo kadhaa hutakiwa kutumia ugonjwa huu mapema na kuanza matibabu.

Lengo ya utafiti:
Utafiti huu umechukuliwa kwa lengo la kutambua ni watoto wanaozaliwa kati ya miezi ishirini na nane na thelathini na nne au wanozaliwa na kilo katika 1,000 gm na 1,500 gm ambao hupatikanwa na huu madhara ya MBD.

Utaratibu:
Watoto watachaguliwa wa kulazwa katika kituo cha watoto, new born unit, na kutambuliwa kulingana na miezi ya kuzaliwa. Baada ya kukubali kushirika katika huu uamuzi, damu itapimwa kama wamefika wiki sita na kufanyiwa uchunguzi. Kilo na mzunguko wa kicha pia utapimwa wakati wa kuzaliwa na baada ya wiki sita. Tutakuomba nambari ya simu ili kukujulisha wakati wa kuleta mtoto apimiwe wakati mtapopatiwa ruhusa kuenda nyumbani.

Sampuli
Sampuli ya damu ya mililiter mbili itachukuliwa kutoka mtoto wako akifika waki sita baada ya mtoto kuzaliwa.

Kufuatilia
Utafiti huu utahitaji samplui kutolewa wakati mtoto amefikisha wiki wa sita baada ya kuzaliwa. Watoto wataopata ruhusa kaabla utafiti haujamalizika watapigiwa simu kwa mwaliisha siku ya kurudi clinic ili sampuli zipiwe.

Faida:
Matokeo ya uchunguzi uliyofanywa itatumika kwa vipasavyo kusimamia mtoto wako. Mtoto wako akipatikana na huu madhara, virutubisho anavyohitaji vitaanzishwa na kuendelea na kufuatiliwa katika clinic. Matokeo ya utafiti huu itatumika kusimamia watoto wote watakatikana na huu madhara. Habari zilizopatikana zitatumika kuboresha huduma kwa hawa watoto ili kuunda hati na inaweza kuchapisha katika majarida ya matibabu na / au iliyothelewa katika makongamano kisayansi (yote ya ndani na kimataifa). Faida kwa washiriki pamoja na elimu ya afya juu ya huduma neonatal. Faida kwa mshiriki itakuwa ni pamoja na masomo ya afya juu ya huduma za watoto wa changa. Faida ya utafiti huu ni pamoja na uwezekano wa kupunguza watoto wanaopatikana na huu ugonjwa

Hadhari:
Uchungu wa maabara unahusisha kuchukua sampuli za damu ya mililita moja ya damu kutoka mtoto wako. Hii itasababisha usumbu wo kidogo kwake. Tahadhari zitachukuliwa kuhakikisha taratibu safi na kuzua kuvunjua damu. Mikakati itachukuliwa kwa washiriki wa utafiti na itafanyika kwa uangalifu kwa hatari ndogo sana au bila hadhari za muda mrefu kwa washiriki.
Kama utaamua kushiriki, unaweza kuondoa wakati wowote bila adhabu au maelezo. Hii kwa vyovyote haitabadilisha mpango wa tiba ambao madaktari wako wanadhani ni nzuri kwa ajili yako, au katika njia nyingine yoyote kuleta chuki kwa mmoja wenu. Matokeo ya utafiti itaangaliwa kwa usiri wa juu, utambulisho wako utatalindwa (jina lako halitatumika na utatambuliwa na nambari, inayojulikana tu na mimi na msaidizi wangu).

SEHEMU YA PILI: Shahada ya Idhini

Nambari Maalum: ……………………………………

Tamko la muhisika:
Mimi __________________________________________ baada ya kupokea taarifa za kutosha kuhusu utafiti, hatari, faida hivyo na KUBALIANA / KUTOKUBALIANA (weka alama iliyofaa) kushiriki katika utafiti na mtoto wangu. Mimi ninaelewa kwamba ushiriki wetu ni kwa hiari na kwamba mimi niko huru kuondoa wakati wowote. Nimepewa fursa ya kutosha kuuliza maswali na kutafuta ufafanuzi juu ya utafiti na wamejibu kuridhisha.

Sahihi ya mazazi: ____________________________ Tarehe ________

Mimi __________________________________________ natangaza kwamba nimeeleza vya kutosha kwa mshiriki hapo juu, utaratibu wa utafiti, hatari, na faida na alizopewa kwake wakati wa kuuliza maswali na kutafuta ufafanuzi kuhusu utafiti. Nimejibu maswali yote ilioulizwa kwa kadri ya uwezo wangu.

Sahihi la mfanyikazi __________________________ Tarehe __________

Kwa ajili ya swali lolote au ufafanuzi, tafadhali usisite kuwasiliana na mimi (Dr. Wambui Wanyoike) kwa +254 719 368 567, au msimamizi wangu Professa Musoke kwa +254 721 307 160, au wasiliana na mwenyekiti wa Maadili kamati, Hospitali ya Taifa ya Kenyatta, P.O BOX 19676-00202, Nairobi Nambari ya Simu (254-020) 2726300 Ext 44355
Appendix 2: Data Collection Form

Study Title: Prevalence of Metabolic Bone Disease of Prematurity using biochemical markers at 6 weeks post-natal age at Kenyatta National Hospital.

Date.......................................................... Serial number ......................................................

Please fill the following patient details from the patient files in the spaces provided.

SECTION A. DEMOGRAPHIC CHARACTERISTICS

1. Sex:  Male [ ]  Female [ ]
2. Gestational age confirmed using the Ballard method: ………. weeks
3. Date of Birth ……………………………
4. Growth Parameters to be measured:

<table>
<thead>
<tr>
<th>Weight (grams)</th>
<th>Head Circumference (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At Birth</td>
<td></td>
</tr>
<tr>
<td>At 6 weeks post-natal age</td>
<td></td>
</tr>
</tbody>
</table>

Please comment if the baby is

[ ] AGA
[ ] SGA
[ ] IUGR

1. Age at which birth weight was regained? ……………….day of life
2. Weight at time of discharge ……………………………….grams
3. Medication history:

<table>
<thead>
<tr>
<th>Medication</th>
<th>Date started</th>
<th>Date stopped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furosemide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminophylline</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1. Feeding history:
When were enteral feeds started? ……………………………days.
Full enteral feed reached at …………days.

<table>
<thead>
<tr>
<th>Day of life</th>
<th>Feeding volumes (mls/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7</td>
<td></td>
</tr>
<tr>
<td>Day 8</td>
<td></td>
</tr>
<tr>
<td>Day 9</td>
<td></td>
</tr>
<tr>
<td>Day 10</td>
<td></td>
</tr>
<tr>
<td>Day 11</td>
<td></td>
</tr>
<tr>
<td>Day 12</td>
<td></td>
</tr>
<tr>
<td>Day 13</td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td></td>
</tr>
</tbody>
</table>

During admission, have enteral feeds ever been interrupted?
Yes [    ] No [    ]
If yes, for how long? Day ………… of life to day ………… of life.
Why were enteral feeds interrupted?
(please state reason or condition)
……………………………………………………………………………………………………………………
……………………………………………………………………………………………………………………
During admission, has the baby been on Partial Parenteral Nutrition?
Yes [    ] No [    ]
If yes, for how long?……………….

1. Medical History:
Are there any conditions the baby developed and was treated for during admission and at what day of life?
Birth asphyxia [    ]
Neonatal sepsis [    ]
Neonatal jaundice [    ]
Necrotizing enterocolitis [  ]
Respiratory distress syndrome [  ]
Other (s) (please specify)………………………………………………………………

2. Kangaroo Mother Care:
Timing of commencement of Kangaroo mother care
Intermittent ……….day of life
Full time ………….day of life

SECTION B: PARENT QUESTIONNAIRE
Please answer the following questions in the spaces provided.
1. Has your child been admitted for any medical condition since discharge from the NBU?
   Yes [  ]    No [  ]
   If yes, for what condition and duration was your child admitted?
   (please specify) …………………………………………………………………………………

Tafadhali jibu maswali yafuatayo kwa nafasi iliyoachwa.
1. Tangu wakati mtoto wako alipopata ruhusa kutoka hospitali, NBU, amewahi lazwa hospitali tena?
   Ndio [  ]    La [  ]
2. Kama ndio, tafadhali eleza sababu ya kulazwa na kwa muda mnapi?

SECTION C: LABORATORY RESULTS

<table>
<thead>
<tr>
<th>Age at sample collection:</th>
<th>Date of sample collection</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>............................days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Appendix 3: Study Budget

<table>
<thead>
<tr>
<th>ITEM</th>
<th>QUANTITY</th>
<th>UNIT COST</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposal development</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Printing</td>
<td>1</td>
<td>8,000</td>
<td>8,000</td>
</tr>
<tr>
<td>Photocopying</td>
<td>4,000</td>
<td>3.00</td>
<td>12,000</td>
</tr>
<tr>
<td>Final proposal booklet</td>
<td>8</td>
<td>500</td>
<td>4,000</td>
</tr>
<tr>
<td>Ethic committee book</td>
<td>1</td>
<td>2000</td>
<td>2,000</td>
</tr>
<tr>
<td>Poster</td>
<td>4</td>
<td>2,500</td>
<td>10,000</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td><strong>36,000</strong></td>
</tr>
<tr>
<td>Stationary</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pens and Pencils</td>
<td>5</td>
<td>20.00</td>
<td>100</td>
</tr>
<tr>
<td>Box files</td>
<td>2</td>
<td>150.00</td>
<td>300</td>
</tr>
<tr>
<td>Spring files</td>
<td>2</td>
<td>120</td>
<td>240</td>
</tr>
<tr>
<td>White out pen</td>
<td>1</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Staple and Staples</td>
<td>1</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Stapler remover</td>
<td>1</td>
<td>235</td>
<td>235</td>
</tr>
<tr>
<td>Paper Punch</td>
<td>1</td>
<td>550</td>
<td>550</td>
</tr>
<tr>
<td>Notebook</td>
<td>2</td>
<td>85</td>
<td>170</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td><strong>2,015</strong></td>
</tr>
<tr>
<td>Clinical and Lab equipment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gloves</td>
<td>5</td>
<td>450</td>
<td>2,250</td>
</tr>
<tr>
<td>ITEM</td>
<td>QUANTITY</td>
<td>UNIT COST</td>
<td>TOTAL</td>
</tr>
<tr>
<td>---------------------------------------------------</td>
<td>----------</td>
<td>-----------</td>
<td>-------</td>
</tr>
<tr>
<td>Syringes (100 pcs)</td>
<td>2</td>
<td>450</td>
<td>900</td>
</tr>
<tr>
<td>Needles (100 pcs)</td>
<td>2</td>
<td>400</td>
<td>800</td>
</tr>
<tr>
<td>Alcohol swabs</td>
<td>2</td>
<td>200</td>
<td>400</td>
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<tr>
<td><strong>TOTAL</strong></td>
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<td></td>
<td><strong>4,350</strong></td>
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<tr>
<td>Laboratory services</td>
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<td></td>
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<tr>
<td>Serum ALP, Corrected Calcium, Phosphorus</td>
<td>104</td>
<td>1,700</td>
<td>176,800</td>
</tr>
<tr>
<td>Sample and result transportation</td>
<td>12 weeks</td>
<td>500</td>
<td>6,000</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td><strong>182,800</strong></td>
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<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Research assistants</td>
<td>2 X 12 weeks</td>
<td>1,500</td>
<td>36,000</td>
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<tr>
<td>Data Statistician</td>
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<td>25,000</td>
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<tr>
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<td>5,000</td>
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<tr>
<td>Computer Services</td>
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<td>5,000</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td><strong>71,000</strong></td>
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<tr>
<td><strong>TOTAL</strong></td>
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### Appendix 4: Time Frame

The following was the time-frame of the study process:

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<thead>
<tr>
<th>Number</th>
<th>Activity</th>
<th>Estimated Time</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Proposal Development and Presentation</td>
<td>4 months</td>
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<tr>
<td>2</td>
<td>Submission of proposal for ethical approval</td>
<td>1 month</td>
</tr>
<tr>
<td>4</td>
<td>Data Collection</td>
<td>5 months</td>
</tr>
<tr>
<td>5</td>
<td>Data Analysis</td>
<td>2 months</td>
</tr>
<tr>
<td>6</td>
<td>Thesis writing</td>
<td>6 months</td>
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<tr>
<td>7</td>
<td>Thesis submission</td>
<td>1 month</td>
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