PREVALENCE OF HYPOCALCEMIA IN INFANTS AGED 1 MONTH TO 6 MONTHS AND ITS ASSOCIATION TO MATERNAL SERUM CALCIUM AT KENYATTA NATIONAL HOSPITAL

A DISSERTATION SUBMITTED IN PART FULFILMENT OF MASTERS OF MEDICINE DEGREE IN PAEDIATRICS AND CHILD HEALTH, UNIVERSITY OF NAIROBI

INVESTIGATOR

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Declaration

This dissertation is my original work, and has not been presented for a degree in any university or published anywhere.

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DEDICATION

To Jasmine.

And to Thomas Theodore, my fleeting angel.

Acknowledgements

God Almighty. Without you none of this would be possible. May your name be praised.

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LIST OF ABBREVIATIONS

Ca – calcium

ATP - adenosine triphosphate

ECF – extracellular fluid

PTH – parathyroid hormone

PTHR – parathyroid hormone receptor

CaR - calcium – sensing receptor

VDR- vitamin D receptor

1, 25(OH) 2D - 1, 25 dihydroxycholecalciferol

PICU – paediatric intensive care unit

KNH – Kenyatta National Hospital

PEU- paediatric Emergency Unit

POPC- paediatric outpatient clinic

QC- quality control

HuQAS- Human quality assessment services

ACRONYMS AND DEFINITION OF TERMS

1. Hypocalcemia- total serum calcium value of less than 2.1 mmol/l.
2. Well baby clinic- clinic for routine immunization and growth monitoring of children under 5 years old

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1.0 ABSTRACT

BACKGROUND

Hypocalcemia occurs as a manifestation of nutritional deficiency of calcium and vitamin D. Deficiency of calcium in the diet has been identified as a leading cause of nutritional rickets in studies from Bangladesh, Nigeria and South Africa. Infants less than 6 months are reliant on mothers for their daily calcium requirements. As such maternal deficiency of calcium may lead to hypocalcemia in the breastfeeding infant.

OBJECTIVES

The primary aim of the study was to establish the prevalence of hypocalcemia in infants aged 1-6 months. The secondary aims were to establish the prevalence of hypocalcemia in the mothers of the infants and to establish if there was an association between the infant and maternal serum calcium.

METHODOLOGY

This was a hospital based descriptive cross-sectional study carried out in the well baby and paediatric outpatient clinics at Kenyatta National Hospital. It was carried out over a period of 3 months. Infants aged 1-6 months and their mothers who fit the defined criteria were recruited for the study. Consecutive sampling was employed till the desired sample size was achieved. A standard questionnaire was administered and venous blood samples collected from the mother-infant pairs. The samples were analyzed for calcium and albumin and corrected calcium levels calculated. Continuous variables were presented as means with standard deviations. Categorical data was presented as percentages. Association between maternal and infant calcium was done using pearson Chi square test.

RESULTS

A total of 120 mother- infant pairs were screened. 94 (78.3%) of these gave samples which were analyzed. The mean age of the infants sampled was 3.5 months with a standard deviation of 1.8 months. The mean age of the mothers was 27.2 years with a standard deviation of 5.8 years.

78 (83%) infants were born at term and 16 infants (17%) were born preterm. 58 of 93 infants (62.4%) were exclusively breastfeeding whereas 35 of 93 infants (37.6%) were breastfeeding but not exclusive.

Prevalence of hypocalcemia was 34% in the infants with 95% CI of 29.4-45%.

Prevalence of hypocalcemia in the mothers of the infants was 39.4% with a 95% CI of 29.8-50.5%.

There was no statistically significant association between hypocalcemia in the mother and infants. OR 1.4 (95% CI 0.6-3.4) and P= 0.447.

CONCLUSION

There is a high prevalence of hypocalcemia in breastfeeding infants as well as in lactating women. However there is no significant association between maternal and infant hypocalcemia

RECCOMMENDATIONS

Advice to mothers to increase dietary intake of Vitamin D and Calcium and to spend more time in the sun. To also ensure their infants spend adequate time in the sun

2.0 BACKGROUND AND LITERATURE REVIEW

2.1 BACKGROUND

Hypocalcaemia is defined as a total serum calcium concentration of < 2.1 mmol/L in children, <2 mmol/L in term neonates and <1.75 mmol/L in preterm neonates. Measurement of ionized calcium is important to distinguish true hypocalcaemia from a mere decrease in total serum calcium. Total serum calcium can be affected by serum albumin levels as well as body pH.(1)

Calcium has a wide range of biological functions in both the ionized form as well as the bound complexes. It also plays an important role in skeletal mineralization. Calcium is an essential element that is only available to the body through dietary sources. (2)

2.11 MEASUREMENT OF SERUM CALCIUM

Less than half of total serum calcium is in ionized form. The remainder is bound to proteins predominantly albumin. As such, in case of hypoalbuminaemia, a low total serum calcium assay will not be a true reflection. Thus if total serum calcium is measured, it is corrected for albumin as follows

Ca(c) = Ca (m) + 0.02(40-measured albumin in g/dl))

Where Ca(c) is the corrected calcium and Ca (m) is the measured calcium.

Ionized calcium is the ideal measurement because it is minimally affected by protein binding and pH but requires assay within 1 hour. This makes it difficult to use it as the measurement in this study.

2.12 SOURCES OF CALCIUM

The foetus in utero and the exclusively breastfeeding infant receive their calcium from the mother via the placenta and breast milk respectively. The best known sources of dietary calcium

include milk and dairy products such as cheese, cream and yoghurt. Non dairy sources include kale and spinach, okra, watercress, kidney beans and soya beans. Bioavailability of calcium in breast milk is higher than of formula- based milks (58% and 38% respectively). As such breast milk is the optimal source of calcium in the first year of life. (4)

2.13 CALCIUM METABOLISM

Calcium must be ionized and in solution for it to be absorbed. It is absorbed by both active and passive transport in the small intestine and a small amount in the large intestine. The bioavailability is dependent on the source and nutritional adequacy. If adequate dietary intake, differences in bioavailability are minimal. In cases of insufficient dietary intake, milk calcium is then more absorbable and has better bioavailability than from other sources. Sources of calcium with high fibre content need to be digested for the calcium to be absorbable. (3).

Once ingested, calcium is absorbed either by passive diffusion or by active transport. Passive diffusion occurs down an electrochemical gradient through intercellular junctions. It accounts for most calcium absorption when intake is adequate or high. It is not saturable, and does not depend on vitamin D either. Factors that decrease its solubility or form complexes such as phytates and oxalates decrease the passive diffusion.

Active transport of calcium is regulated by dietary intake and body needs. It is a saturable mechanism localized to the duodenum and is dependent on vitamin D. Calcitriol, the active metabolite of Vitamin D influences several steps in this active transport. Its most striking effect is its control of the expression of the gene encoding calcium binding protein, thereby regulating

the migration of calcium across intestinal cells. It also increases membrane permeability and activates the Ca-ATPase.

Most of the calcium in the body is found in the bone. 99% of total body calcium is present in the skeleton as calcium complexes, primarily as hydroxyapatite. (2). It provides skeletal strength and a dynamic pool for maintaining intra and extracellular calcium concentration.

In the ECF, calcium circulates in 3 fractions: about 50% is the biologically important ionized fraction, 40% is protein-bound and 10% is complexed to anions such as bicarbonate, citrate, sulphate, phosphate, and lactate. Most of the protein-bound calcium is bound to albumin, the remainder being complexed to globulins.

Disorders that lower serum albumin will lower total serum calcium but have a lesser effect on the ionised calcium concentration. Binding of calcium to albumin is also affected by ECF pH. Acidaemia will decrease protein binding and increase the ionised calcium. For each 0·1 decrease in pH ionized calcium rises by about 0·05 mmol/L.(5)

Calcium homeostasis is largely regulated through an integrated hormonal system that controls calcium transport in the gut, kidney, and bone. It involves the parathyroid hormone (PTH), vitamin D as well as serum ionized calcium and the calcium-sensing receptor (CaR).

A decrease in serum calcium inactivates the CaR in the parathyroid glands to increase PTH secretion, which acts on the PTHR in kidney to increase tubular calcium reabsorption, and in bone to increase net bone resorption. The increased PTH also stimulates the kidney to increase secretion of 1, 25(OH)2D, which activates the VDR in gut to increase calcium absorption, in the parathyroid glands to decrease PTH secretion, and in bone to increase resorption. The decrease in serum calcium probably also inactivates the CaR in kidney to increase calcium reabsorption and

potentiate the effect of PTH. This integrated hormonal response restores serum calcium and closes the negative feedback loop. Together, these negative feedback mechanisms help to maintain total serum calcium levels in healthy individuals within a relatively narrow physiologic range.(2)

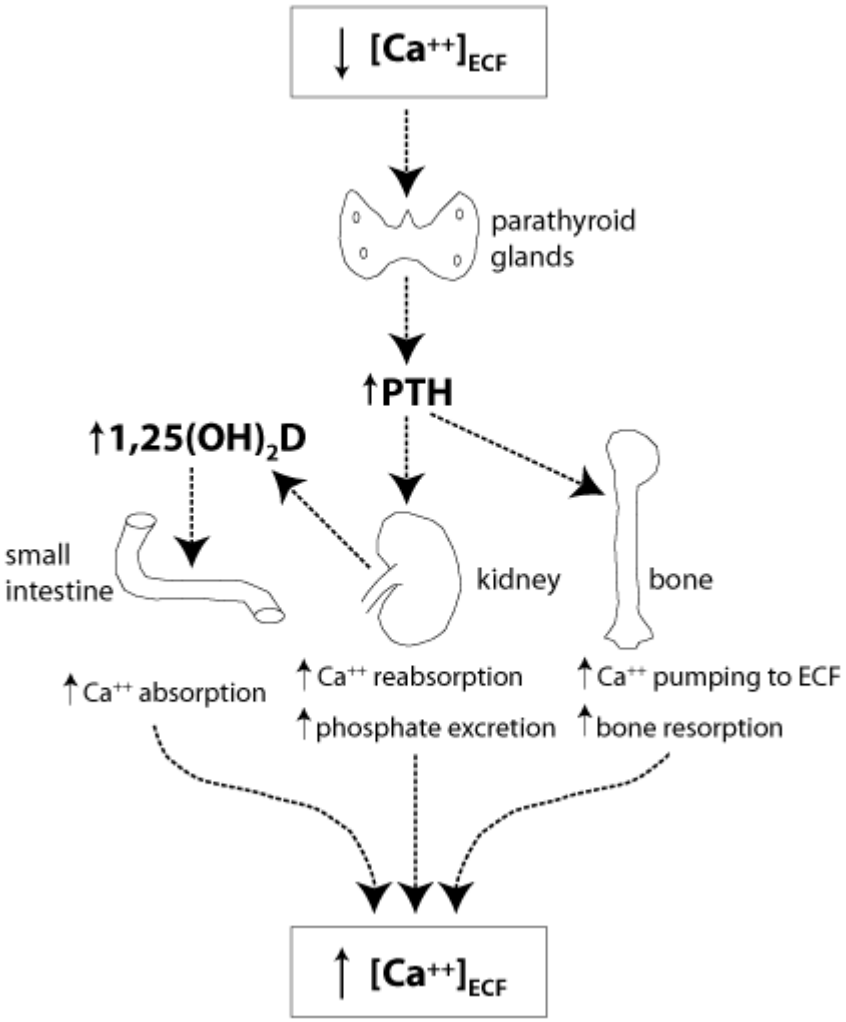


Figure1. Calcium homeostasis

2.2 LITERATURE REVIEW

2.21 PREVALENCE STUDIES

There are few prevalence studies of hypocalcemia..

A retrospective study done in Yemen by Bin Mohanna et al (6) found a prevalence of 58% in a hospital based setting. Ogunkolo in Nigeria(7) found a prevalence of 11.53% in patients randomly selected from patients that walked into a Clinical Biochemistry laboratory in a University Teaching Hospital and Singi in India(8) found a prevalence of hypocalcemia of 35% in critically ill children in paediatric intensive care unit (PICU).

Bin Mohanna and colleagues performed a retrospective study of patients seen at a specialized paediatric center in Sana'a city, Yemen. Records for 90600 patients aged 0-4 years seen during a 4 year time period of 1999- 2003 were reviewed. They found that 310 of the cases seen at the time were suspected to have hypocalcemia and subjected to serum calcium assays. The findings from the study were as follows: prevalence of hypocalcemia of 58% (Chi- square = 1.9 and p= 0.17). The findings were also presented based on age groups as follows. 17.8% in those 0-1months, 17.2% in those 1-3 months and 25% in those aged 3-6 months.

Ogunkolo et al in Nigeria randomly picked 902 participants from patients presenting at the clinical biochemistry laboratory in Olabisi Onabanju University Teaching hospital for various reasons. They assayed the total serum calcium and albumin levels of the participants and calculated the corrected calcium levels. The prevalence of hypocalcemia was found to be 11.53%

Locally Dr. Kisiangani in his Mmed dissertation (9) found a prevalence of neonatal hypocalcaemia of 21.5% with significant correlation with maternal calcium levels p=0.013. The

significance of mentioning this is that both the exclusively breastfeeding infant 1-6 months and the foetus in utero rely on the mother 100% for their calcium requirements.

TABLE 1: SUMMARY OF PREVALENCE STUDIES

Name, year	Country	Study site	Study population	Study size	Hypocalcemia prevalence
BinMohanna et al, 2005	Yemen	Specialized pediatric hospital	0-4 years	310	58%. Age-specific:17.8% in 0-1months, 17.2% in 1-3months, 25% in 3-6 months
Ogunkolo et al,2006	Nigeria	University Teaching hospital	Not indicated	902	11.53%
Singhi et al, 2003	India	Pediatric Intensive Care unit	6 mo- 12 years	100	35%
Kisiangani Mmed dissertation, 2011	Kenya	National Referral Hospital	0-28 days	121	21.5%in neonates, 29% n mothers

2.22 CAUSES OF HYPOCALCEMIA

Studies show several causes of hypocalcemia. Nutritional deficiencies of calcium and vitamin D are some of the commonest causes of hypocalcemia.

Hypoparathyroidism leads to hypocalcaemia by 2 mechanisms. First, the loss of PTH results in a failure of renal 1,25 dihydroxyvitamin D [1,25$(OH)_2$D] production, with a resultant reduction in the ability to absorb dietary calcium. Second, PTH is a potent anticalciuric agent in the distal convoluted tubule. Therefore, its loss results in increases in renal calcium excretion (10)

Hypomagnesaemia with magnesium depletion causes impaired synthesis or secretion of parathyroid hormone. This impairment would account for the hypocalcemia observed in the hypomagnesemic state. (11)

Several factors may contribute to the hypocalcemia of acute renal failure. Among these are hyperphosphatemia, hypomagnesemia and magnesium depletion, hypoalbuminaemia or alterations in calcium binding by serum proteins, failure of parathyroid gland function and skeletal resistance to the action of parathyroid hormone. (12).

Ionized hypocalcemia has multifactorial causes in critically ill patients especially with sepsis. This includes hypomagnesaemia, elevated circulating cytokines leading to Hypoparathyroidism. Other causes include defective vitamin D absorption and activation. (13)

2.23 HYPOCALCEMIA AND NUTRITIONAL DEFECIENCIES

Daily calcium requirements vary with age. Infants from birth to 6 months require 210mg/day of elemental calcium intake. Those aged 7 to 12 months require 270mg /day. Children aged 1to 2 years require 500mg /day whereas older children 4-8 years require up to 800mg/day and adults require up to 1200mg per day. (14)

Studies from Kenya by Neumann C and Bwibo NO on micronutrient intake found daily calcium intake from 24 hr dietary recall of 316mg/day. This was from both the diet at home and that given in school. The total daily calcium intake is well below recommended daily requirements for children and adults. (15**)** This is significant in that if the child is taking that amount from the family diet, then it means all family members including the mothers are getting similar amounts. As such their body stores are not adequate.

The foetus in utero and exclusively breastfeeding infants are solely dependent on the mother for their calcium requirements. Depletion of maternal calcium and vitamin D stores will affect calcium status in the foetus and infant and this can lead to hypocalcaemia. (16)

Thatcher T. et al concluded from a study in Nigeria that reduced breast-milk calcium concentration may contribute to a reduced calcium intake in infancy thus predisposing the infants to hypocalcaemia and nutritional rickets. They conducted a case control study of 35 women who were currently breastfeeding and had other children on follow up for rickets. For each case mother, 3 control mothers were selected from matched mothers within the same stage of lactation. The mothers' breast milk was assayed for calcium levels. The mean breast milk calcium concentration of mothers of children with rickets was less than that of control mothers P=0.034. (17)

Deficiency of calcium is one of the causes of nutritional rickets. Studies from Nigeria (18) and Bangladesh (19) identify calcium as a significant cause of nutritional rickets the studies found that the subjects had low dietary intake of calcium with significant response to calcium therapy.

2.24 EFFECTS OF HYPOCALCAEMIA

There are several effects of hypocalcemia. These include nutritional rickets which is associated with increased incidences of respiratory infection, of which pneumonia is included. (20). Cardiac performance is demonstrably reduced by hypocalcaemia, with decreased myocardial contractility and hence decreased left ventricular stroke work index, ejection fraction and cardiac index**.** This can lead to reversible heart failure. (21). Other cardiovascular effects include dilated cardiomyopathy (22) and even cardiogenic shock (23).

Acute hypocalcaemia causes increased peripheral neuromuscular irritability. This can lead to mild symptoms such as paraesthesia or muscle cramps to severe symptoms such as tetany (24)

and laryngospasms with stridor . (25, 26)

Hypocalcaemia is associated with various types of seizures including grand mal, petit mal and focal seizures. The presence of seizures without tetany in patients with hypocalcaemia may be explained by the observation that low cerebrospinal fluid ionized calcium concentrations may have a convulsive effect. (27)**.**

Three types of dermatological changes have been described in hypocalcaemic patients (1) alopecia, transverse growing of the nails, and edema; (2) dry, scaly, pigmented skin, scanty hair growth, and onychorrhexis; (3) moniliasis, especially in idiopathic hypoparathyroidism.(28)

3.0 STUDY JUSTIFICATION AND UTILITY

The prevalence of rickets is on the increase worldwide

Low intake of calcium is one of the causes of rickets which may manifest with hypocalcaemia. Maternal dietary deficiency of calcium may lead to infant calcium derangements and nutritional rickets.

Studies have shown that rickets is associated with increased respiratory infections. Pneumonia is one of the top 5 causes of under- 5 mortality rate. Measures to reduce nutritional rickets will help reduce pneumonia and thus the mortality rate.

The study will ascertain need to supplement calcium in breastfeeding women and hence reduce the prevalence of nutritional rickets in the infant. It will also form a basis on which further studies related to calcium homeostasis can be done.

4.0 RESEARCH QUESTION

What is the prevalence of hypocalcaemia in breastfeeding infants aged 1month to 6 months and what is the association with maternal serum calcium levels.

4.1 STUDY OBJECTIVES

4.1.1 Primary objective

. To find out prevalence of hypocalcaemia in breastfeeding infants aged 1 month to 6 months.

4.1.2 Secondary objectives

1. To find out prevalence of hypocalcaemia in the mothers of the above infants

2. To establish the association between serum calcium levels in mothers and their breastfeeding infants aged 1 to 6 months.

5.0 STUDYMETHODS

5.1 STUDY DESIGN

Hospital based cross sectional survey.

5.2 STUDY SITE

Participants were recruited from the well baby clinic, the paediatric emergency unit (PEU) and the paediatric outpatients follow up clinics (POPC) at Kenyatta National hospital. Kenyatta National Hospital is a national teaching and referral hospital located in Nairobi Kenya.

The well baby clinic is run Monday to Friday from 9 am to 3 pm. This is where well babies are followed up for routine childhood immunization and follow up. Other than vaccinations for the children admitted in the wards, the clinic sees about 250 children in a month. The relatively low numbers are due to the fact that most mothers are advised to go to the nearest clinics for immunization at discharge.

The paediatric outpatient clinic is run every Thursday from 2-4 pm. All children discharged from the wards are reviewed in the clinic for follow up. On average around 150 children are reviewed every week.

The paediatric emergency unit is the 1st point of entry for all sick children aged less than 13 years. Some of the sick children are admitted while most are seen as outpatients and go home on oral medications. On average about 5000 children are seen here every month.

5.3 STUDY POPULATION

5.3.1 Inclusion criteria

Infants aged 1 to 6 months born preterm and term and currently breastfeeding

Written informed consent

5.3.2 Exclusion criteria

Declined consent

Renal disease in mother or infant

Liver disease in mother or infant

Mothers with metabolic bone disease or known hypoparathyroidism

Sick infants admitted or going for admission in pediatric wards

5.4 SAMPLING

Consecutive sampling of all eligible participants was applied. The sample size was calculated using Fischer's formula as shown

$$n = \frac{Z^2 p(1-p)}{d^2}$$

Where

n = sample size

(Z1-α)2 *=two-sided significance level . (95% CI)= 1.96*

P = estimated prevalence or proportion (used 21 % based on Dr. Kisiangani findings)

d = precision error (taken as 0.08) -8%

Sample size of 100

STUDY PROCEDURE

Recruitment and training of research assistants

One research assistant was recruited and trained. Training included research methodology. The purpose and aim of the study was explained to the research assistant. The study objectives, the inclusion and exclusion criteria were explained to her. We went through the structured questionnaire as a data collection tool. Any questions and concerns about how to collect the information were sorted out. Instructions on the sample collection were given and appropriate labeling explained. The research assistant was also educated on research ethical issues related to research with human subjects and human samples and were be expected to uphold them throughout the study period. She signed a confidentiality agreement a sample of which is attached on appendix V.

Once training was complete, a dry run was performed to establish if the research tool and procedure were well understood and followed. Any problems picked up during the dry run were handled accordingly

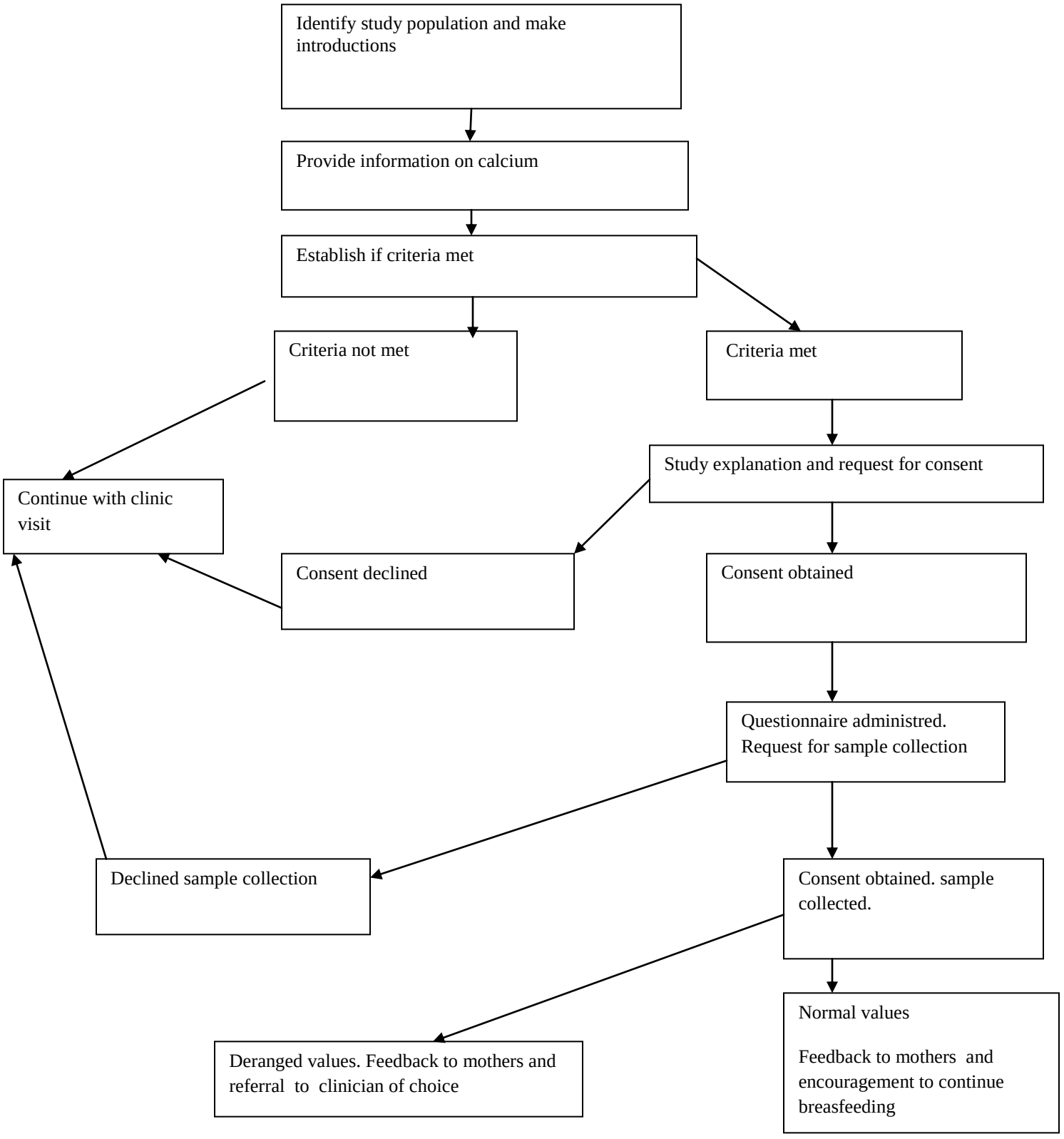
Study tools

The study tool used was a structured questionnaire. This was a 5 part structured questionnaire that included infant details, maternal details dietary details and a section for putting in maternal and infant calcium levels. It was filled by the mothers with assistance from the research assistant where necessary. It was in English.

Study location

The study was conducted at the PEU, POPC and well baby clinics at KNH

STUDY PROCEDURE



Screening and recruitment

There were daily visits to the relevant clinics during the duration of the clinics coverage. Potential participants were identified, approached and introductions made. The mother was then requested to follow the investigator to the prepared room for a brief chat. If a father was present then he too was invited for the talk. Once settled in the secluded room, the purpose of the study was explained to the potential participants. Potential risks and benefits involved were explained. Eligible clients who meet the inclusion criteria were included and informed written and signed consent form (in English or Kiswahili depending on participants' preference and fluency) were sought. This was countersigned by the principal investigator. If the potential participant had one of the exclusion criteria or declined to participate, they were thanked for their time and released. Consecutive sampling where all children meeting the criteria were included till the required sample size was obtained.

The questionnaire was then administered with assisted completion if participants were illiterate or unable to understand English. For follow up and referral purposes if necessary, the study link log was filled to capture the contact details of the participants. It contained the study serial number and contacts. Appendix II and IV. This was only be accessed by the research assistant during data collection and the principal investigator.

The specimens were collected aseptically. 3ml of venous blood was collected from the mother and 3 ml from the infant. The collected blood was stored in plain labeled bottles. The samples were transferred to the lab within 2 hours of collection. Studies show that samples for calcium and albumin are stable for up to 72 hours when stored at room temperature of 23-25 degrees C. (30)

Consecutive sampling was done during the times we were in the clinics

Laboratory analysis

The samples were processed at the renal lab for Kenyatta National Hospital. The Mindray BS 400 machine was used to assay calcium and albumin levels. The samples were first centrifuged to separate the plasma. This was then placed in the analyzer machine. This is a fully automated machine that works on the principal of photometry.

The machines are calibrated when the QC is off or when new reagents are inserted into the machines. By virtue of the large numbers served reagents are changed and thus calibration done every 3rd day. Internal quality control (QC) is done once daily or when the machine raises an alarm. External QC is done at 2 levels. Every fortnight an interlaboratory QC is done. The labs involved are the 4 biochemistry labs at KNH. The 2nd level of external QC is done every 3 months by the Human Quality Assessment Services (HuQAS).

The machines are serviced when need arises. This is when the QC is off the expected range. In the last 1 year, the machines were serviced 3 times. This was done by servicemen from the Mindray Company, manufacturers of the machine.

The samples were analyzed for calcium and albumin. The corrected calcium was then calculated as follows:

Ca(c) = Ca (m) + 0.02(40-measured albumin in g/dl))

Where Ca(c) is the corrected calcium and Ca (m) is the measured calcium.

Maintenance of confidentiality

Care was taken to maintain the confidentiality of the participants. The samples were collected in a secluded area. The questionnaire had only a serial number and other biodata. No identifiers were on the questionnaire. For purposes of follow up and referral there was a link log containing

only the study serial number, the childs 1st name and the contacts of the mother. This was only accessed by the research assistant during data collection and the principal investigator. Laboratory request forms from KNH were used. They only contained the study serial number and either maternal sample or infants sample as identifiers. The results were collected daily from the lab. All documents pertaining to the study were kept in a locked cabinet. They will be promptly be incinerated once the study is completed successfully. As part of maintenance of confidentiality, the research assistant signed a confidentially agreement and was expected to keep it.

6.0. TIME FRAME

The study took 3 months between December 2013 and February 2014

7.0 DATA MANAGEMENT AND ANALYSIS

Data was coded from the questionnaires and entered into Microsoft Access database. Data cleaning was performed prior to data analysis to ensure errors during data entry were corrected. Data analysis was done using SPSS version 17.0.

The study population was described using the socio-demographic and nutritional/dietary characteristics. Continuous variables such as age were presented as means with standard deviations. Categorical data such as sex, education level, occupation, and child's feeding option and dietary descriptions of the mother were analyzed and presented as percentages. Prevalence of hypocalcemia with its 95% confidence interval was analyzed and presented as proportion of all the studied population. Further, linear regression was done to show how mother's calcium levels predict the levels of calcium in their infants. Hypocalcemia was associated for both infant and the mother using Pearson Chi square test.

8.0 ETHICAL CONSIDERATIONS

Approval for the study was sought from the Kenyatta National hospital and university of Nairobi scientific and ethics committee. Two study assistants were recruited and trained to understand the objectives and procedures of the study. This was a qualified clinical officers from the pediatric department. I introduced the study assistants to the administration and staff of the clinics. Signed consent was sought from the eligible clients before enrolling them in the study. The consent form is in appendix. The participants were subjected to any extra cost because of the study. Women and children found to be deficient of calcium were contacted and referred to the appropriate clinics for supplementation and follow up.

9.0 RESULTS

The results are presented starting from a brief summary of the sampled population. This is followed by the social and demographic characteristics of the study population. This is followed by the results of calcium findings in the infants and in the mothers. Finally are the results of the factors associated with infant hypocalcemia.

A total of 120 mother- infant pairs were screened. Of these, 12 did not meet the criteria and were not recruited. Of the remaining 108, 10 refused to sign consent. 4 signed consent but declined sample collection. This left a balance of 94 for analysis. This was 78.3 % of all those screened. Of the screened, 11.6% met the inclusion and exclusion criteria but declined either to sign consent or to have samples collected

SOCIO-DEMOGRAPHIC CHARACTERISTICS OF STUDY POPULATION

The mean age of the enrolled children was 3.5 months with a standard deviation of 1.8. The mean age of the mother was 27.2 years with a standard deviation of 5.8. 78 children enrolled (83%) were born at term whereas 16 infants (17%) were born preterm.

2 mothers (2.1%) had no education, 37 (39.4%) had completed primary education,31 (33%) had completed secondary and 24 (25.5%) had completed tertiary level of education.

53 mothers (56.4%) were unemployed, 13 (13.8%) were employed in formal sector and 28(29.8%) were self employed.

The mean number of household occupants was 4.1 members with a SD of 1.4

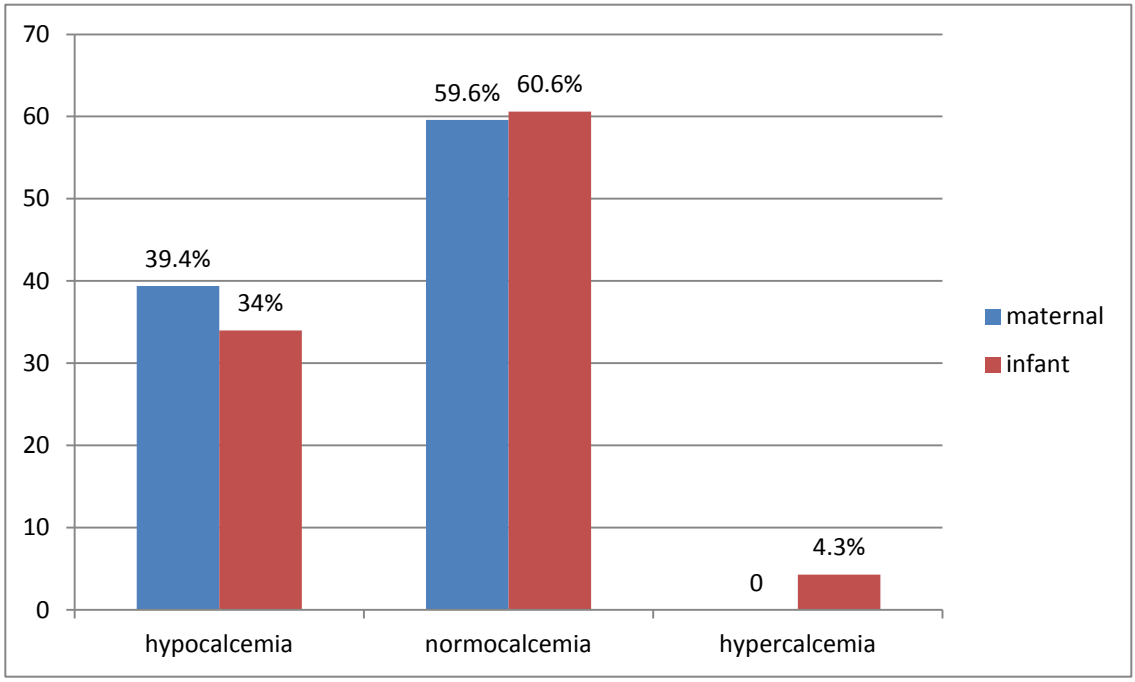
TABLE 2: SOCIAL AND DEMOGRAPHIC CHARACTERISTICS

Characteristic	Frequency (%) or mean (SD)
Infant's age (months)	3.5 (1.8)
Gestation age of the child at birth Term Preterm	 78 (83.0) 16 (17.0)
Maternal age (years)	27.2 (5.8)
Level of maternal education None Primary Secondary Tertiary	 2 (2.1) 37 (39.4) 31 (33.0) 24 (25.5)
Maternal symptoms in preceding 3 months Numbness around the mouth Twitching Muscle spasms	 8 (8.5) 0 (0.0) 5 (5.3)
Number of household occupants	4.1 (1.4)

PREVALENCE OF HYPOCALCEMIA

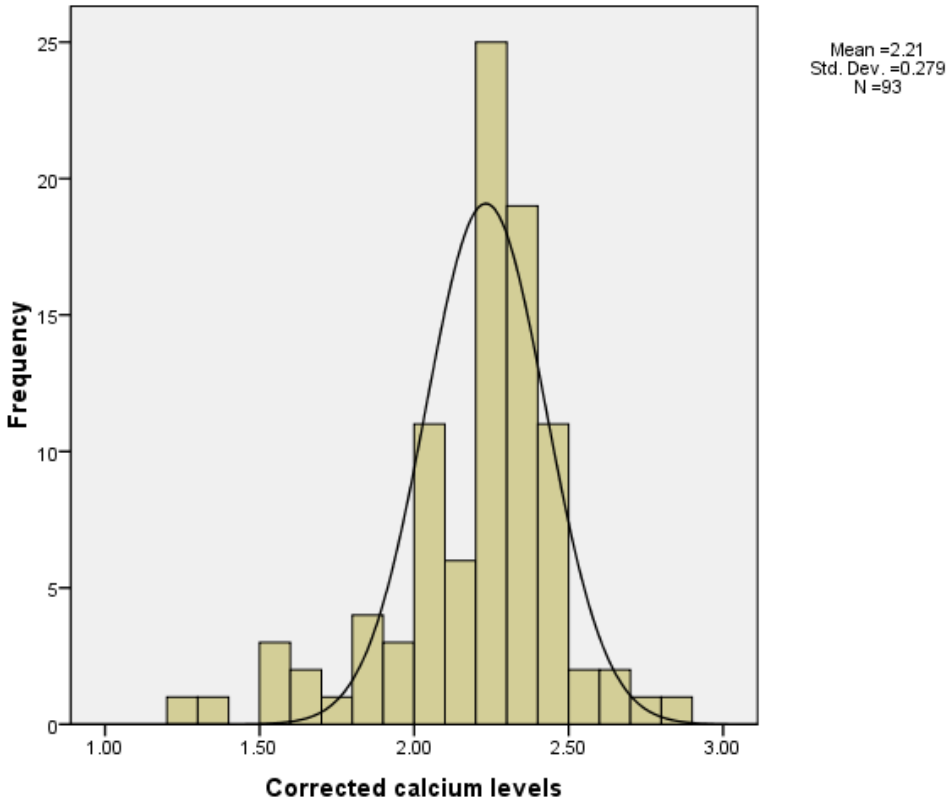
32 out of 93 infants (34%) had hypocalcemia with 57 (59.6%) having normal calcium and 4(4.3%) had hypercalcemia. The 95% confidence interval for the corrected calcium in the infant was 29.4-45%. 37 out of 93 mothers (39.4%) had hypocalcemia (CI 29.8-50.5%). 56 mothers (59.6%) had normal calcium.

FIGURE 2 : CORRECTED CALCIUM LEVELS



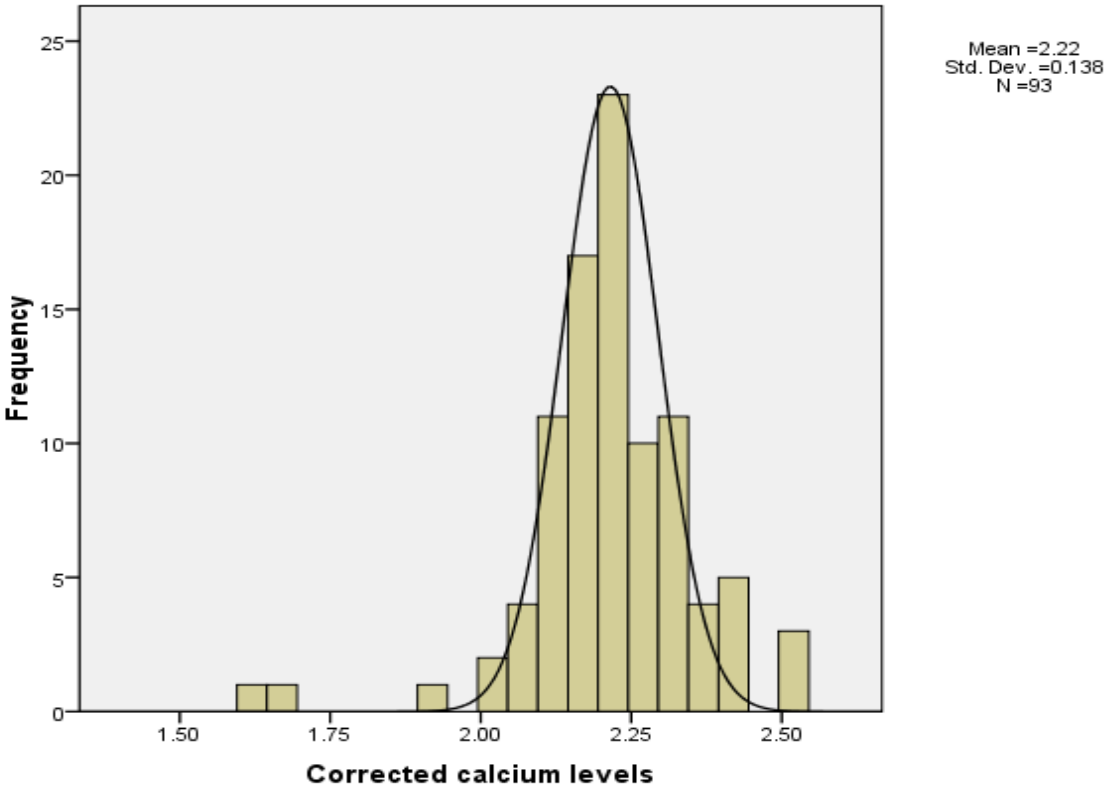
The mean calcium corrected calcium for the infant was 2.21mmol/L with a standard deviation of 0.279.

FIGURE 3: NORMOGRAM OF CORRECTED CALCIUM IN INFANT



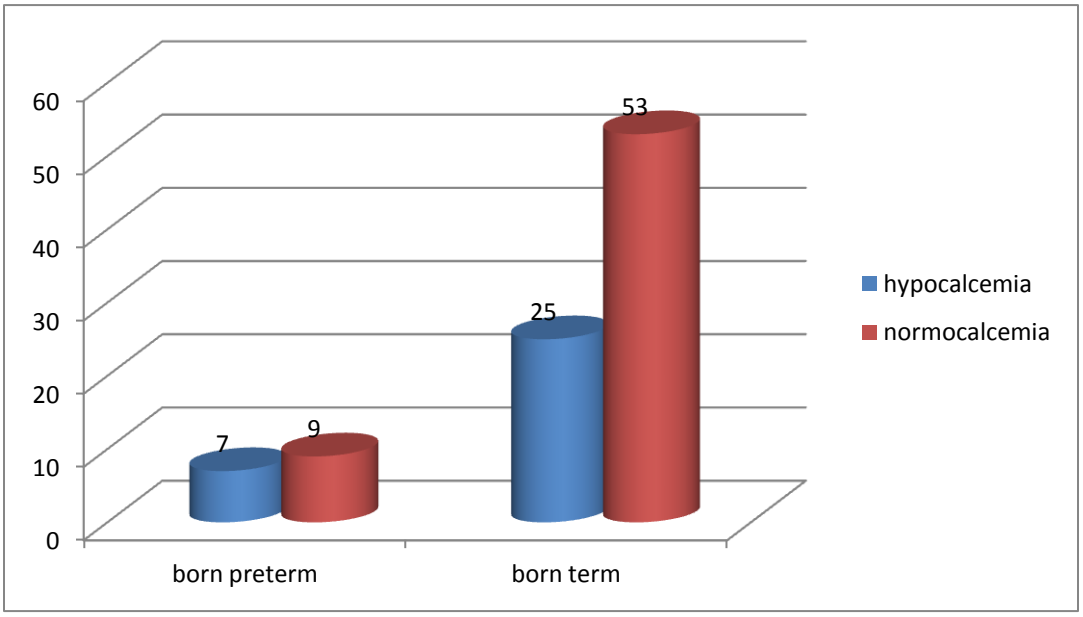
The mean corrected calcium for the mother was 2.22mmol/L with a standard deviation of 0.138.

FIGURE 4: NORMOGRAM OF CORRECTED CALCIUM IN MOTHER



Of the infants recruited, 7 out of the 16 infants born preterm (44%) had hypocalcemia compared to 15 out of 78 of the infants born term (32%). P = 0.37 Odds Ratio 1.65 with a 95% confidence interval 0.55- 4.94

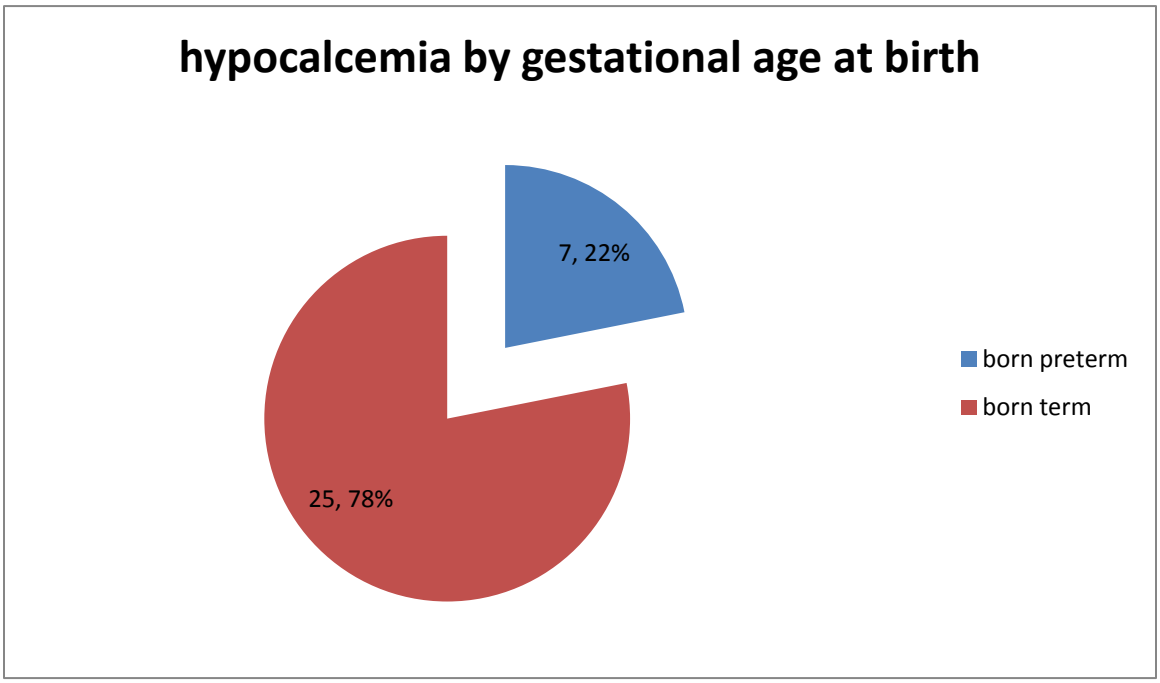
FIGURE 5: CALCIUM LEVELS OF INFANTS BASED ON GESTATION AGE AT BIRTH



Of the 32 infants with hypocalcemia, 7 (21.9%) were born preterm and 25 (78.1%) were born term.

FIGURE 6: HYPOCALCEMIA BY GESTATIONAL AGE AT BIRTH

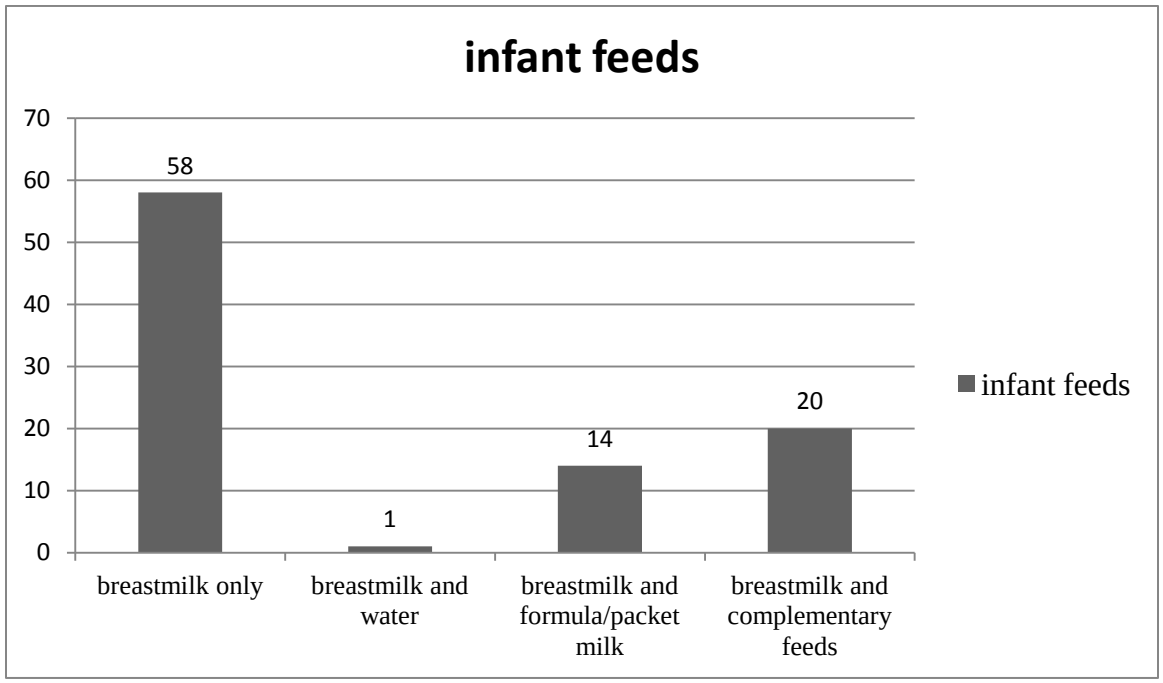
N=32



The main feed of the infants recruited was exclusive breastfeeding. 58 out of 93 infants (62.4%) were on exclusive breastfeeding. 14 out of 93 infants (15.1 %)were on breast milk and formula and 20 out of 93 infants (21.5%) were already on complementary feeds.

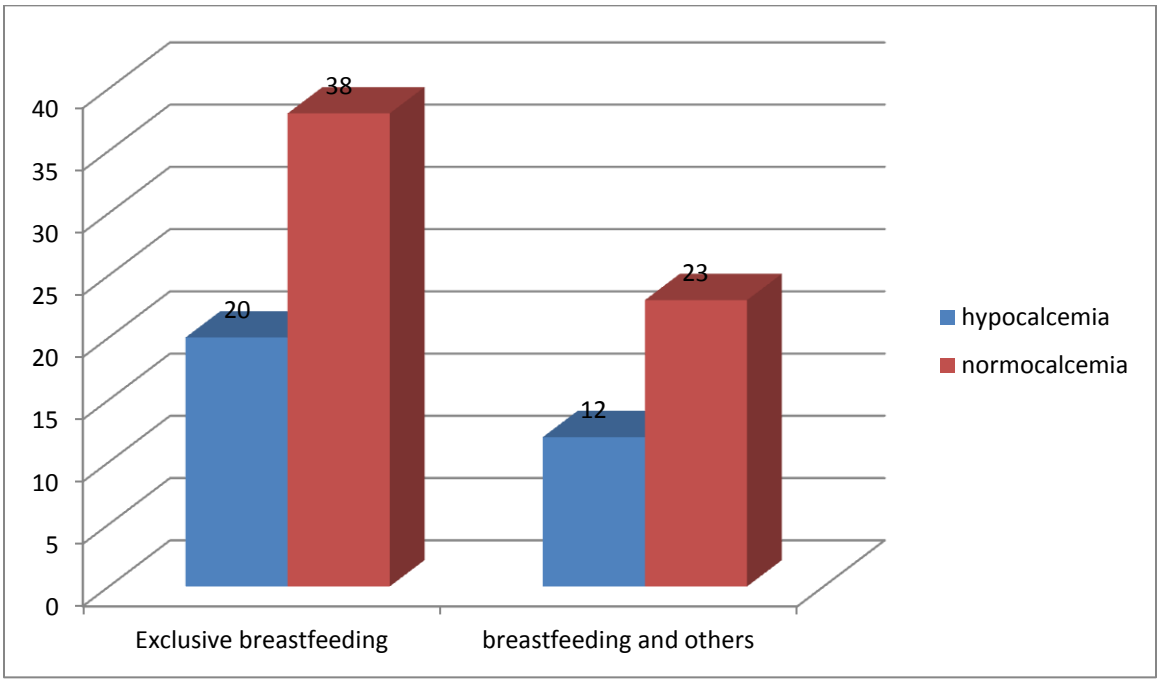
FIGURE 7: INFANT FEEDS

N= 93



Of the infants recruited, 20 out of 58 exclusively breastfeeding infants (34.5%) had hypocalcemia compared to 12 out of the 35 infants (34.3%) who were not exclusively breastfeeding OR 1.0 , 95% CI 0.4-2.4

FIGURE 9: CALCIUM LEVELS BASED ON INFANTS FEEDS



In the 24 hr dietary recall, only 50 (53.2%) of the mothers had taken milk in the 24 hrs prior. Only 2 (2.1%) only had taken fish and 25 (26.6%) had taken eggs.

Of the 93 mothers, 70 (74.5%) prepared their cereal by boiling before soaking nad only 23(24.5%) soaked the beans before boiling.

Milk intake by the mothers was such that 31.9% took milk daily, 33% took milk less than twice a month.

TABLE 3: MATERNAL DIETARY AND PHYSICAL HABITS.

DIETARY /PHYSICAL HABITS	Frequency (%)
Food content in preceding 24 hrs	
Milk	50 (53.2)
Fish	2 (2.1)
Eggs	25 (26.6)
Cereal preparation	
Boiled without soaking	70 (74.5)
Soaked before boiling	23 (24.5)
Frequency of taking milk	
Daily	30 (31.9)
At least twice a week	19 (20.2)
Once a week	14 (14.9)
Less than twice a month	31 (33.0)
Frequency of taking eggs	
Daily	5 (5.3)
At least twice a week	41 (43.6)
Once a week	25 (25.5)
Twice a month	11 (11.7)
Once a month	10 (10.6)
Missing	3 (3.2)
Beverage prepared for the whole household	
Yes	91 (96.8)
No	3 (3.2)
Packets of milk used in the household in a day	
None	2 (2.1)
1-2	74 (78.7)
3-4	15 (16.0)
More than5	2 (2.1)
Missing	1 (1.1)
Use of other dairy products (n=72)	72 (76.6)
Daily	4 (5.6)
1-2 times a week	28 (38.9)
1-2 times a month	40 (55.6)
Time spent in the sun	
n=90	51 (56.7)
Less than one hour	38 (42.2)
More than one hour	1 (1.1)
Once a week	

There was no statistically significant association between the infant hypocalcemia and the maternal hypocalcemia. This was same as with the other variables measured as shown in table 4.

TABLE 4: FACTORS ASSOCIATED WITH INFANT HYPOCALCEMIA

factor		Hypocalcaemia	Normal calcium	OR (95%)	P value
Mothers calcium levels	Hypocalcaemia Normal	15 (40.5) 17 (32.7)	22 (59.5) 35 (67.3)	1.4 (0.6-3.4) 1.0	0.447
Spend time in the sun	No Yes	1 (33.3) 31 (36.0)	2 (66.7) 55 (64.0)	0.9 (0.1-10.2) 1.0	0.923
Boiled without soaking	No Yes	9 (39.1) 23 (35.4)	14 (60.9) 42 (64.6)	1.2 (0.4-3.1) 1.0	0.748
Soaked before boiling	No Yes	19 (35.8) 8 (34.8)	34 (64.2) 15 (65.2)	1.0 (0.4-2.9) 1.0	0.929
Using milk	No Yes	12 (29.3) 20 (41.7)	29 (70.7) 28 (58.3)	0.6 (0.2-1.4) 1.0	0.224
Using other dairy products	No Yes	6 (30.0) 25 (36.8)	14 (70.0) 43 (63.2)	0.7 (0.3-2.2) 1.0	0.578
Using eggs	No Yes	21 (32.3) 11 (45.8)	44 (67.7) 13 (54.2)	0.6 (0.2-1.5) 1.0	0.238
Using fish	No Yes	32 (36.8) 0 (0.0)	55 (63.2) 2 (100.0)	- 	0.284
Exclusive breast feeding	No Yes	12 (34.3) 20 (37.0)	23 (65.7) 34 (63.0)	0.9 (0.4-2.2) 1.0	0.792

10.0 DISCUSSION

In this study we found a high prevalence of hypocalcemia using the laboratory ranges of 2.2-2.6mmol/l. the prevalence in the infants was 34% with a 95% CI of 29.5- 45.5% with an equally high prevalence in the mothers of 39.4%. CI 29.8-50.5%.

The large confidence interval is attributed to use of a precision error of 8 % which gave a smaller sample size but a larger confidence interval. As such the prevalence we found while not being very precise, gives as an idea of what the value is like.

Other factors that could contribute to high reported prevalence are the inclusion of preterm in the study sample. The infants born preterm contributed 17% of the study population. Preterms are a unique population prone to rickets of prematurity due to inadequate transfers of maternal vitamin D which mainly occurs in the 3^{rd} trimester. Of the 32 children with hypocalcemia 7 of them (21.9%) were born preterm and 25(78.1%) were born term.

However, 7 of 16 born preterm (44%) compared to 25 of 78 born term (32%) had hypocalcemia which contributed to the high prevalence of hypocalcemia in the infants.

Inclusion of some sick infants at the pediatric emergency and pediatric outpatient clinic could also have contributed to the noted high prevalence. In as much as none of the children had a clinical diagnosis of rickets, it is likely that due to illness and derangement of hormonal balance, hypocalcemia was noted.

That being said, the prevalence found of 34% is comparable to the prevalence found by Singi et al in India(8). Singi found a prevalence of hypocalcemia of 35 % in children admitted in a PICU. The similarity ends there as in the study from India, children in PICU were on management severe illness and sepsis is known to cause hypocalcemia.

Ogunkolo in Nigeria found a prevalence of hypocalcemia of 11.53% in an unspecified study population. It is impossible to make a comparison between the 2 studies due to because the study population in Nigeria is not defined.

Lailou et al in a survey in Vietnam found a prevalence of mild hypocalcemia of 97% in young children aged < 5 years in 19 provinces in Vietnam. He also found a prevalence of hypocalcemia of 83% in the general population of women of child bearing age. The recorded prevalence values from the general population are quite high and were found to be due to dietary deficiency of both calcium and vitamin D. The values are much higher than those obtained during our study. The study population is ideal as it has taken into account the general population .(30)

The infant feeding mode did not seem to affect calcium levels in the infants much. There was no significant difference in hypocalcemia between the infants who were exclusively breastfed and those who were not. Although this presumption is prejudiced by the lack of power in the sample size of each.

In the maternal dietary intake, we had a 24 hr dietary recall. Only 50 % of the mothers had taken a diet rich in calcium and only 2.1% had taken fish and 26.6% had taken eggs. This shown low dietary intake by the mothers of both calcium and vitamin D in the previous 24 hrs. Apart from that, the frequency of taking eggs is relatively low. 10.6% of sampled population took eggs only once a month and 43.6 took eggs at least once a week. As far as milk is concerned, most mothers made tea for the whole household and majority used 1-2 packets of milk. Each packet of milk is 500 mls and contains 212 mg of calcium/100ml. this leads to a low dietary intake of calcium.

Also notable is 74.5% of the sampled mothers prepared their cereals without soaking. This means their meals had quite a high phytate content which leads to inhibition of calcium

absorption. Despite this, there is no significant association hypocalcemia in the infant and the household where cereal was prepared before soaking. OR 1.2 and a p value of 0.748

56.7% of the sampled mothers spend less than 1 hour in the sun every day. Only 42.2% spend more than an hour daily in the sun and 1 mother responded to spending time in the sun only once a week. This is insufficient time to get adequate vitamin D stores both for the mother and for her breastfeeding infants.

Thus likely that there is a high prevalence of hypocalcemia in the mothers and infants due to lack of both calcium and vitamin D due to both dietary and physical habits of the mother.

Despite these findings, in our study did not find any statistically significant association between hypocalcemia in the infant with any of the variables measured.

STUDY LIMITATIONS

1. Inclusion of preterm infants in study increases measured prevalence and thus affects results as preterm are predisposed to rickets of prematurity due to inadequate or absent transfer of calcium and vitamin D from the mother. Transfer of calcium and phosphorus mainly occurs in the 3rd trimester

2. Inclusion of children on follow up in pediatric outpatient clinic and the pediatric emergency unit may have skewed the results found. The children may have had unidentified chronic illnesses that affect calcium metabolism and as such contributed to the high prevalence in infants

3. The exclusion of known liver disease was part of the criteria. This however did not account for the mothers or children with undiagnosed liver disease. As such inclusion of some infants who had low albumin levels may have affected the results

4. The exclusion criteria did not exclude children with malnutrition. These children may have low albumin levels due to inadequate protein intake

5. Many mothers give children unprescribed multivitamins which may contain vitamin d. I did not include this in the exclusion criteria or the questionnaire. This wound affect the prevalence of calcium measured as vitamin D increases calcium absorption

6. Measurement of serum calcium may not be best modality of assessing body calcium stores as it's a late manifestation and calcium is tightly controlled by hormones. Bone density would be a better one

7. A number of the questions required the mother to have a 24 hour recall. This could introduce recall bias

CONCLUSION

There is a high prevalence of hypocalcemia in breastfeeding infants and in lactating women. There is however no significant correlation between hypocalcemia in the mothers and in the breastfeeding infants.

RECOMMENDATIONS

1. Advice to breastfeeding mothers to spend time in the sun as well as to ensure their infants spend time in the sun
2. Consider evaluating calcium and vitamin D supplementation in mothers and breastfeeding infants.
3. In-depth studies to establish the local reasons for the high prevalence of hypocalcemia in the mothers and their infants

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APPENDIX 1

INFORMED CONSENT FORM (ENGLISH)

I am Dr. Jacqueline Lichuma, a post graduate student from the department of Paediatrics and Child health at the University of Nairobi.

I am carrying out a study as part of my postgraduate training titled 'PREVALENCE OF HYPOCALCEMIA IN BREASTFEEDING INFANTS AGED 1 MONTH TO 6 MONTHS AND ITS CORRELATION TO MATERNAL SERUM CALCIUM AT KENYATTA NATIONAL HOSPITAL'.

Calcium is very important for you and your baby. Your exclusively breastfeeding baby receives all the calcium from breast milk. Calcium is important for making strong bones and teeth and many other functions in the body. Albumin is a protein made by the liver that combines with calcium. It is important to measure it as well as it can affect the level of calcium recorded.

The study will involve answering some questions about the child, you and home environment. I will also need to collect some blood 3-5 mls from you and your baby. This will be to check for the levels of calcium and albumin in your blood and in the babies' blood. No other tests will be done on the collected sample. In case of any deficiency, we will contact you and give you a referral to a facility of your choice for further care.

Your participation is voluntary with no rewards to you financial or otherwise. You are free to participate or withdraw from the study at any time. Participation is not a pre condition to receiving services in this institution now or in future. The study will be done at no added cost to you. Any questions you have regarding calcium will be answered.

If you have any questions regarding the study you can contact me the principal investigator Dr. Jacqueline Lichuma on number 0723261124. If any questions on your rights as a participant, please contact Kenyatta national Hospital Ethics and Research Committee by calling 2726300 ext 44355.

I, _____ having received adequate information about the study hereby agree to participate in the study with my child. I understand that our participation is voluntary and that I am free to withdraw at any point.

Mother's signature: _____ Date: _____

Interviewer's signature: _____ Date: _____

TAFSIRI: IDHINI YA KUSHIRIKI KATIKA UTAFITI (KISWAHILI)

Mimi Daktari Jacqueline Lichuma kutoka Chuo Kikuu cha Nairobi, masomo ya afya ya watoto. Kuambatana na masomo yangu, ninafanya uchunguzi unaoitwa - 'PREVALENCE OF HYPOCALCEMIA IN BREASTFEEDING INFANTS AGED 1 MONTH TO 6 MONTHS AND ITS CORRELATION TO MATERNAL SERUM CALCIUM AT KENYATTA NATIONAL HOSPITAL '

Madini ya CALCIUM ni muhimu sana kwako na kwa mtoto wako anayenyonya. Mtoto anayenyonya bila kupata chakula chochote kingine anapata madini haya ya CALCIUM kutoka kwa maziwa yako.CALCIUM inasaidia kutengeneza mifupa na meno yenye nguvu. Albumin ni madini yanayotengenezwa na maini yako. Albumin hushikana na calcium na inaweza kupotosha kiwango cha calcium kinachopimwa.

Utafiti unahitaji wewe kujibu maswali kadhaa. Baadaye kiwango kidogo (3-5mls) cha damu kutoka kwako na kwa mtoto kitachukuliwa ili kupima madini ya CALCIUM na albumin. Hakuna kipimo kingine kitapimwa. Tukipata kuna upungufu wa madini haya kwako ama kwa mtoto nitakuarifu na kukutuma kwa hospitali ambayo unataka ili kusuluisha upungufu huo.

Ushiriki wako katika utafiti huu ni kwa hiari yako bila kulazimishwa na bila malipo yoyote. Uko huru kushiriki na pia kutoka kwa utafiti huu wakati wowote. Kutoshiriki kwako hakutakuzuia kupata usaidizi katika hospitali hii sasa na hata wakati ujao. Ukikubali kushiriki, hutatoa malipo yoyote ya ziada. Maswali yako yote kuhusu madini ya CALCIUM yatajibiwa.

Ukiwa na maswali yoyote kuhusu utafiti huu, unaweza kunipata Dr. Jacqueline Lichuma kwa nambari ya simu 0723261124. Ukiwa na maswali yoyote kuhusu mahitaji yako kama mhusishwa wa utafiti unaweza piga simu kwa Kenyatta National Hospital Ethics and Research Committee kwa nambari ya simu 2726300 ext 44355.

Ninaomba idhini ya kwa kushiriki kwako wewe na mtoto wako.

RUHUSA.

Mimi _____ mzazi wa _____ nimeelewa maelezo juu ya utafiti huu na ninakubali mtoto wangu kushiriki.

Sahihi ya mzazi _____ Tarehe _____

Sahihi ya mchunguzi _____ Tarehe _____

APPENDIX II.

QUESTIONNAIRE

PREVALENCE OF HYPOCALCEMIA IN BREASTFEEDING INFANTS AGED 1 MONTH TO 6 MONTHS AND ITS CORRELATION TO MATERNAL SERUM CALCIUM AT KENYATTA NATIONAL HOSPITAL.

PART 1

Date: _____

Study serial no_____

Age of child in months: _____ weeks_____

PART 2 INFANT FACTORS

1. Child born at what gestational age

    1. Term
    2. Preterm

    If preterm, at what gestational age _____

2. What does the baby feed on?

    1. Breast milk only

    2. Breast milk and water

    3. Breast milk and formula/packet milk

    4. Breast milk and others

        List other feeds given_____

5. Is the child on calcium supplements

1. Yes

2. No

PART 3

1. Maternal age : _____
2. Level of education : _____
3. Occupation:_____
4. Residence: _____
5. Household occupants count: _____

6. Have you ever taken calcium as a supplement?

1. Yes

2. No

7. Have you been taking anticonvulsants in the last three months?

1. Yes

2. No

8. Have you experienced any of the following in the last three months?

a. Numbness around the mouth: 1.yes_____ 2. no_____
b. Twitching: 1. yes_____ 2. no _____
c. Muscle spasms: 1. yes ___ 2. no_____

9. Do you spend time in the sun? 1. Yes ___ 2. No ____

If so, how much time?

d. Less than 1 hour in a day: 1. yes____ 2. No____
e. More than 1 hour daily: 1. yes. ____ 2.no____
f. Once a week: 1. yes. ____ 2.no____
g. Once a month: 1. yes. ____ 2.no____

PART 4: DIETARY RECALL**.**

Have you taken any of the following in the last 24 hours?

1. Milk: 1.yes____ 2. No_____
2. Fish: 1.yes_____ 2. No_____
3. Eggs: 1. Yes___ 2. No_____

Cereals and legumes

1. Beans: 1.yes____ 2. No_____
2. Green grams: 1. Yes_____ 2.no. _____
3. Soya 1. Yes_____ 2.no _____

Cereal preparation

1. Boiled without soaking: 1. Yes_____2. no. _____
2. soaked before boiling: 1. Yes_____2._ no._____
3. Fermenting: 1.yes_____2. no_____
4. Sprouting: 1. Yes_____2. no _____

6. How many times do you take the following?

a). Milk: 1. Daily

2. At least twice a week

3. Once a week

4. Twice a month

5. Less than twice a month

b). Eggs: 1. Daily

2. At least twice a week

3. Once a week

4. Twice a month

5. Once a month

7. Is your beverage (tea, cocoa, and coffee) prepared with for the whole household?

1. Yes

2. No

8. How many packets of milk do your household use in a day?

a. none
b. 1-2
c. 3-4
d. More than 5

9. Do you take other dairy products such as yoghurt, fermented meal, cheese

1. Yes

2. No

10. if yes to number 9, how frequently ?

a. daily

b. 1-2 times a week

c. 1-2 times a month

PART 5: LAB FINDINGS

Total calcium level:

Mother: _____

Baby: _____

Serum Albumin

Mother _____

Baby_____

Corrected Calcium

Mother _____

Baby _____

APPENDIX III

REFERRAL LETTER

NAME:

AGE:

I/P NO:

RE: REFERRAL FOR FURTHER MANAGEMENT

I am Dr. Lichuma C. Jacqueline, carrying out a study on the prevalence of hypocalcaemia and correlation to maternal calcium of infants aged 1 month to 6 months at Kenyatta National Hospital and Mbagathi District hospital. During the study the above named participant was found to be hypocalcaemic and is therefore referred for urgent attention.

Attached please find a copy of the participants' laboratory findings.

Yours sincerely,

Dr. Lichuma C. J

APPENDIX IV

STUDY LINK LOG

PREVALENCE OF HYPOCALCEMIA IN BREASTFEEDING INFANTS AGED 1 MONTH TO 6 MONTHS AND ITS CORRELATION TO MATERNAL SERUM CALCIUM AT KENYATTA NATIONAL HOSPITAL

PREVALENCE OF HYPOCALCEMIA AND ITS CORRELATION TO MATERNAL CALCIUM IN BREASTFEEDING INFANTS AGED 1 TO 6 MONTHS

STUDY SERIAL NUMBER	CHILD'S NAME	MOTHER'S CONTACT NUMBER
1		
2		
3		
.		
.		
.		
130		

APPENDIX V

CONFIDENTIALITY AGREEMENT

STUDY: **PREVALENCE OF HYPOCALCEMIA IN BREASTFEEDING INFANTS AGED 1 MONTH TO 6 MONTHS AND ITS CORRELATION TO MATERNAL SERUM CALCIUM AT KENYATTA NATIONAL HOSPITAL**

Principal investigator: Dr. Jacqueline Lichuma

Research assistant: _____

I, having been explained to and understanding the ethical issues related to research with human subjects, do promise to maintain confidentiality while participation in this research. All information obtained from the participants will be discussed with the principal investigator only. All data collected during the study period will be promptly handed over to the principal investigator.

Signature _____

Date _____

Id number_____

APPENDIX VI

BUDGET

	ITEM	COST (KSHS)
1.	STATIONERY	4000
2.	PRINTING AND BINDING	10000
3.	RESEARCH ASSISTANTS (1)	12000
4.	STATISTICIAN	30000
5.	LABORATORY INVESTIGATIONS	300000
6.	CONTIGENCY	20000
	TOTAL	376000

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

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke

Link:www.uonbi.ac.ke/activities/KNHUoN

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

Ref: KNH-ERC/A/316

10th October 2013

Dr. Lichuma Jacqueline
Dept.of Paediatrics &Child Health
School of Medicine
University of Nairobi

Dear Dr. Lichuma

RESEARCH PROPOSAL: PREVLAENCE OF HYPOCALCEMIA IN BREASTFEEDING INFANTS AGED 1 MONTH TO 6 MONTHS AND ITS CORRELATION TO MATERNAL SERUM CALCIUM AT KENYATTA NATIONAL HOSPITAL (P214/05/2013)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 10th October 2013 to 9th October 2014.

This approval is subject to compliance with the following requirements:

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

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

"Protect to Discover"

Yours sincerely

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

c.c. Prof. A.N.Guantai, Chairperson, KNH/UoN-ERC
The Deputy Director CS, KNH
The Principal, College of Health Sciences, UoN
The Dean, School of Medicine, UoN
The Chairman, dept. of Paediatircs &Child Health, UoN
The Assistant Director, Health Information, KNH
Supervisors: Prof. Nimrod Bwibo, Dr. Lucy Mungai