VITAMIN D STATUS AND MAGNESIUM STATUS AMONG INFANTS AGED 6 WEEKS TO 12 MONTHS ATTENDING CHUKA COUNTY REFERRAL HOSPITAL WELL BABY CLINIC.

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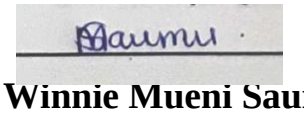
2023

DECLARATION

I solemnly confirm that this thesis is my original work and to the best of my knowledge has not been submitted elsewhere for examination

Signature:  **Date:** 23th November, 2023

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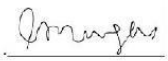
Signature:  **Date:** November 23, 2023

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DEDICATION

I gladly dedicate this work to my beloved husband, Dr. Paul Bundi Karau, and our three Children; Bundi Karau Junior, Maluki Bundi and Muriira Bundi.

I sincerely thank you for walking this journey with me. May God bless you.

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SUMMARY

Background: Vitamin D deficiency is a re- emerging public health problem across the globe. Vitamin D plays a key role in calcium absorption, bone mineralization and aids in phosphate and magnesium metabolism. Early life vitamin D deficiency is associated with adverse child health outcomes. This deficiency increases the risk of stunted growth, rickets, recurrent respiratory infections, atopy, type 1 diabetes and cardiovascular risk later in life. Magnesium deficiency has been shown to reduce vitamin D levels. There are few studies done in urban settings that reveal a high prevalence of vitamin D deficiency among infants. The magnitude of co- existence of vitamin D and magnesium deficiency remains under- explored in Kenya.

Objective: To determine the status of vitamin D among infant- mother pairs, Magnesium and other related biochemical parameters among infants attending the routine well-baby clinic at Chuka County Referral Hospital.

Methodology: This was a descriptive cross-sectional study. To find qualified volunteers, we applied sequential sampling. For the caregivers who consented, obtained data on the infants' and caregivers' sociodemographic and infants' clinical characteristics.

We drew 3 mls of mother's peripheral blood to determine vitamin D levels and 3 mls of infant's peripheral blood to determine vitamin D, magnesium, parathyroid hormone, calcium, phosphorus and alkaline phosphatase levels. The Kenyatta National Hospital/University of Nairobi Ethics Review Committee and Chuka Hospital Management granted the ethical approval for the study.

Data Analysis: IBM version 21.0 SPSS was used for the analysis of the data. We made use of descriptive statistics to analyse the levels of measured variables in the serum.

Results: A total of 122 infant mother pairs were recruited with 56 % male, 44% females and a mean age of 5.5 months. Majority of the infants were born at term via SVD. 58 % reported sun exposure for >30 minutes and majority were exclusively breastfed. 87.7% of the caregivers were from low socio-economic status with secondary school education. The mean serum Vitamin D levels among infants and their mothers was 52.57ng/dl and 37.95ng/dl. We found vitamin D deficiency and insufficiency among infants to be 5% and 6.5% respectively. 9% and 22% of mothers were vitamin D deficient and insufficient respectively. All infants were magnesium sufficient. There was a significant linear correlation between infant and maternal Vitamin D levels ($r = 0.285$, $P= 0.002$).

Conclusion: Hypovitaminosis D is still a major problem in the tropics. Policies on adequate sun exposure and vitamin D supplementation among breastfeeding mothers and their infants should be considered.

1.0 INTRODUCTION

One of the most prevalent nutritional problems in children is lack of vitamin D, 25(OH)D. An estimated 50% of people worldwide suffer from undernourishment as a result of micronutrient deficiencies (Norman et al., 1997). Depending on laboratory cut-offs, studies in Africa have revealed a 34-59% general occurrence of deficiency of vitamin D ((Bikle, 2014).

Ergocalciferol-D2 and cholecalciferol-D3, the source compounds of 25(OH)D, are inactive molecules that must be sequentially hydroxylated by the hepatic and renal systems to become the active form ((Bikle, 2014). Early-life deficiency of vitamin D is linked to dietary variables, inadequate sunlight exposure, and growing demands for nutrients for children's growth and development.

With its deficiency, there comes a higher likelihood of rickets, growth retardation, respiratory infections, allergic diseases, and type 1 diabetes. Lack of vitamin D throughout childhood has been linked to adult malignancies, cardiovascular disease, and susceptibility to contagious infections (Zisi et al., 2019), (Buonsenso et al., 2018) , (Agarwal et al., 2015).

Together, calcium (Ca), magnesium (Mg), and phosphorus (P) are thought to make up 98%, 65%, and 80% of a person's skeletal weight, respectively. The majority of calcium and phosphorus are present in conjunction as portions of the bone component apatite crystals [Ca5(PO4)3(OH), which only make the bone when calcium and phosphorus are present in the right ratios (Underland et al., 2020).

Magnesium is often deficient or suboptimal in many weaning diets for infants. Magnesium is

crucial in activating 25 hydroxy Vitamin D to 1,25, hydroxyl Vitamin D. Given the crucial part magnesium plays in sustaining infants' bone health, more research is required.

Nutritional calcium is essential for bone calcification from early childhood through adolescence. Vitamin D status and nutrition can have an impact on calcium levels. Significant calcium intake deficits probably harm peak skeletal mass accrual and raise the chances of long-term loss of bone mass. The parathyroid hormone controls the bioactivity of vitamin D and related hormones (Khundmiri et al., 2016)

There are few studies documenting serum phosphorus levels in well infants, despite its critical role in skeletal development and integrity. Whereas vitamin D promotes calcium absorption, it promotes the excretion of phosphorus, thereby maintaining the balance needed for skeletal integrity.

Studies on vitamin D, as well as other bone biochemical parameters among infants, have been carried out in major urban areas in Kenya (Karuri et al., 2017); (Said et al., 2020). Despite Magnesium and vitamin D's importance in early childhood development and the benefits of timely supplementation, there is a dearth of literature on vitamin D and Magnesium levels among healthy infants in rural Kenya.

2.0 LITERATURE REVIEW

2.1 Prevalence of Vitamin D and Magnesium Deficiency among infants

The incidence of lack of vitamin D, 25(OH)D, has been progressively increasing in recent years, with the majority of studies revealing mixed evidence. About twelve per cent of Boston residents, in a given study, for instance, were 25(OH)D deficient (Gordon et al., 2008)

An aggregated prevalence of 17.31 per cent was reported in Africa by a recent meta-analysis. Compared to populations residing in Sub-Saharan Africa, South Africa and some countries in northern Africa revealed the highest prevalence rates in a study by (Mogire et al., 2020). There was 81% prevalence of 25(OH)D deficiency in a South African cohort of newborns aged 6 to 10 weeks, which is fairly high (Ncayiyana et al., 2021). In comparison, a previous study in the same location indicated an incidence of 33 per cent (Velaphi et al., 2019). Recent research on Tanzanian infants revealed prevalence rates of 25(OH)D insufficiency and deficiency of 16.5 per cent and 22 per cent respectively. This seems to be similar to the figures Said et al. (2020) reported among urban Kenyan infants who were exclusively breastfed (Said et al., 2020). Stella Karuri et al. examined discharge information of children aged between one month to five years in three locations within Kenya and discovered that rickets was diagnosed in 4.01% and 0.92% of the children in the capital and central part of Kenya, respectively (Karuri et al., 2017).

There are few studies on prevalence of Magnesium deficiency among infants. A study done in India showed a prevalence of 44.8% among infants (Dandinavar et al., 2019)

2.2 Biochemistry of Vitamin D

The skin synthesizes Vitamin D3 in a process that involves breakdown of aromatic rings by UV light from the sun by a non-enzymatic process from 7-dehydrocholesterol (7-DHC). Pre-cholecalciferol is then synthesized to cholecalciferol in a thermo-responsive but non-catalytic process (Holick et al., 1980). Diet-sourced 25(OH)D is also available. Ergosterol in plants and fungi is exposed to UVB radiation to create vitamin D2 (ergocalciferol). It differs from cholecalciferol in that the side chain has a carboxyl group at C24 with a double bond connecting C22 and C23 (Holick et al., 1980). Vitamin D2 has a decreased affinity for vitamin D-protein transport complex as a result of these changes. It is also removed from circulation more rapidly.

Cytochrome P450 mixed-function oxidases carry out the three major phases of the metabolism of vitamin D, namely 25-hydroxylation, 24-hydroxylation, and 1a-hydroxylation. Some of these enzymes, like CYP2R1, are found inside the endoplasmic reticulum (ER), while others, like CYP27A1, CYP27B1, and CYP24A1, are found in the mitochondria (Sugimoto & Shiro, 2012). The majority of 25-hydroxylases come from the liver, where both mitochondria and microsomes have been found (Norman et al., 1997). To synthesise 1, 25 dihydroxy vitamin D, 1- hydroxylase further hydrolyses 25-hydroxy vitamin D in the kidney (Bikle, 2014).

VDR, a key transcription factor that is a member of the steroidal nuclear receptor family of hormones, mediates the genomic effects of 1, 25 dihydroxy vitamin D. In order to carry out its genomic function, which can be specific both at the genetic and cellular levels, VDR attaches to the Vitamin D Receptor Element (VDRE) and attracts the co-regulatory complexes necessary. This makes it possible for 1, 25(OH)2D to behave in a cell type-specific manner. Some vitamin D effects happen too quickly to entail genetic activity. The

earliest of these, known as transcaltachia, included the rapid increase of gastrointestinal Ca transportation in a 25(OH)D- rich chick (Norman et al., 1997).

2.3 Risk factors for low vitamin D

2.3.1 Maternal Vitamin D Status

Maternal 25(OH)D status and child and newborn 25(OH)2D levels are correlated. The human placenta delivers 25(OH)D to the foetus (Kovacs, 2008). Internationally, this has been demonstrated in the US(Bodnar et al., 2007) and Germany (Wuertz et al., 2013). In research conducted in Kenya amongst infant-mother couples, newborns born to women lacking vitamin D had a greater frequency of the condition (Atandi, 2018).

Infantile VDD is avoided by maternal supplementation during pregnancy. Antenatal maternal supplements significantly increased 25(OH)D in blood within the umbilical cord, as reported by a meta-analysis of 43 randomised clinical trials (Roth et al., 2017). A cohort study of American moms who received prenatal vitamin supplements revealed a favourable connection between

a mother's vitamin D levels and her newborn's 25(OH)D. After four months, though, this association was no longer apparent (Merewood et al., 2010). These results were comparable to those of an Australian cross-sectional study conducted among 12-month-olds, which discovered that self-reported antenatal supplement use was not linked to a lower likelihood of VDD (Suaini et al., 2014). This finding demonstrates that a mother’s use of micronutrient supplements during the antenatal period is not protective beyond six months.

Additionally, maternal consumption of vitamin D-rich foods lowers the incidence of 25(OH)D deficiency in neonates and nursing infants. Most foods are fortified with vitamin D in

developed nations. In the US, adult intake within their diets (144-288 IU/day) is higher as a result of enhanced fortification (Bailey et al., 2010); however, it still only amounts to 24-48% of the dietary recommendations limit (Ross et al., 2011).

2.3.2 Exclusive breastfeeding (EBF)/ Complementary feeding

Formula use or use of fortified feeds reduces the risk of 25(OH)D insufficiency. Compared to EBF, mixed feeding and exclusive formula use are linked to a lower risk of 25(OH)D deficiency (Suaini et al., 2014). Similar results were observed by Carpenter et al. Formula-based diets throughout the initial half of the first year of life was associated with greater levels of 25(OH)D. (Carpenter et al., 2013). These findings support the observational research that connects 25(OH)D insufficiency and exclusive breastfeeding. Complementary feeding in foods deficient of vitamin d or not fortified with Vitamin D is a risk factor.

The Bristol Breastfeeding assessment tool

The Bristol breastfeeding assessment tool is a concise breastfeeding assessment tool facilitating accurate rapid breastfeeding appraisal. It helps in assessing the breastfeeding skills in mothers. This tool assesses 4 elements of breastfeeding that include; positioning, attachment, suckling and swallowing. Each element is graded from 0-2 with zero being poor skill and 2 good skill. Mothers with a total score less than seven require more education and support. Accurate assessment is essential to ensure enhanced breastfeeding efficiency and increased maternal self-confidence. The tool can be used both clinically and in research to target advice to improve breast feeding efficacy (Ingram et al., 2002)

2.3.3. UV Exposure and Seasonal Variation

While UV exposure measurements vary between investigations, a link between UV and vitamin D levels has been found to exist (Grant et al., 2009; Merewood et al., 2010; Suaini et al., 2014). According to Merewood et al., children under the age of four months have a higher risk of insufficiency when they spend less than one day per week outside for at least ten minutes (Merewood et al., 2012). In comparison, Grant et al. found no link tying the number of hours six- month-old to two-year-old infants spent outside or donning sunscreen or a hat to VDD in an investigation in New Zealand (Grant et al., 2009). However, the researchers did find that less skin-exposing clothing raised the incidence of VDD. Use of sunscreens increases the risk.

Definition of adequate sun exposure

A minimum of five days per week with at least thirty minutes of sunlight exposure to the face, back of the hands, forearm, and part of the trunk between the hours of 10 a.m. and 4 p.m. has been considered enough sun exposure ((Tangpricha, 2015).

2.3.4 Geographic Factors

Through latitude, geography can influence vitamin D levels directly. Modelling has shown that, given similar circumstances (season, colour of skin, skin exposure, and hours invested outside), the amount of vitamin D generated by a person in Darwin (latitude 12 S) is eight-fold more than that synthesized by a person in Hobart (latitude 42 S). According to the research by Siafarikas et al., average serum vitamin D levels were greater in the south compared to the north in East Germany (Siafarikas et al., 2017).

Local health policies can also have an impact on 25(OH)D status, and some international standards (such as those from the US, UK, Europe, and Canada) advise supplementing under

one-year-old infants with 400 IU of 25(OH)D (Arundel et al., 2012; Grossman et al., 2017). All exclusively breastfed newborns in Australia who have one or more additional risk factors for insufficiency receive supplementation (Roh et al., 2016). These risk factors include having darker complexions (Fitzpatrick type V and type VI), having less time out in the sun due to cultural customs or living in extremely southern climates, or having illnesses that impact how the body processes fat (e.g., cystic fibrosis). The only group for whom supplementation of vitamin D is mandated in Kenya is preterm newborns.

2.3.5 Socioeconomic Status

Extensive research has evaluated the connection between socioeconomic level and 25(OH)D status (Camargo et al., 2010; Grant et al., 2009). Using the New Zealand Deprivation Index, Camargo et al. discovered that individuals with greater levels of wealth exhibited better average 25(OH)D levels than those with lower wealth.

2.3.6 Gestational age

Low gestational age is associated with low vitamin D levels. Generally, the fetus is unable to produce endogenous 25-OH D and is completely dependent on transfer from the mother (Weissman et al., 2003). Preterm infants are at higher risk of vitamin D deficiency as most transplacental transmission of vitan D happens during the third trimester (Kaushal & Magon, 2013). Burris. H et al demonstrated that infants born before 32 weeks gestations had higher odds of vitamin D deficiency than term neonates. This results were echoed by a study done by adnan et al who found that 53% of preterms were vitamin D insufficient. (McCarthy et al., 2013)

2.3.7 Chronic illnesses in the infant

Children with chronic illnesses are predisposed to vitamin D deficiency. In a cross-sectional register based Finnish study, 47% of children with chronic illnesses were found to be vitamin D deficient. (Holmlund-Suila et al., 2013) These children were on follow up for several chronic illnesses including asthma, allergies, gastrointestinal diseases, cancer, renal diseases, diabetes, metabolic bone diseases and chronic infectious diseases.

2.3.8 Maternal use of Chronic medication and contraceptives

A number of drugs are known to interfere with the vitamin D metabolism through upregulation of 25-hydroxyvitamin D_3-24-hydroxylase (CYP24) gene expression and activation of the pregnane X receptor. CYP24 is a mitochondrial enzyme responsible for inactivating vitamin D and thereby causing vitamin D deficiency. (Pascussi et al., 2005) Mothers on treatment for tuberculosis using a rifampicin based regimen are predisposed to vitamin D deficiency. Other drugs that have been implicated include antiretroviral drugs and anti- epileptics such as phenobarbital, phenytoin and carbamazepine . (Gröber et al., 2011) Dexamethasone is one of the drugs given to pregnant women at increased risk of premature labour to accelerate fetal lung maturity and prevent respiratory distress syndrome. It's use induces vitamin D deficiency in mothers which translates to deficiency in their newborns. (Mirzaei et al., 2018)

Use of oral contraceptives is associated with high levels of maternal vitamin D levels. (Ciebiera et al., 2019)

2.3.9 Maternal use of Sunscreens/ skin lightening creams

A sunscreen is a sun protection compound applied to the skin, which should block or absorb radiation in the UV range 290–400 nm, in order to reduce the dose of UV that reaches the skin's surface. The UV filters in sunscreens may be physical or chemical agents. Two physical, inorganic agents are used: titanium dioxide, which provides strong UV-B and some UV-A protection, and zinc oxide, which provides good UV-A and UV-B protection. Most of the chemical, organic agents absorb UV-B radiation, but only a few offer UV-A protection(Glaser & Tomecki, 2020) . In Nothern India Dasgupta A et al found that sunscreen use among pregnant women was srongly correlated with low vitamin D levels which translated to low vitamin D levels in their newborns(Dasgupta et al., 2012). Light skinned individuals and those who use skin lightening creams require less exposure to sunlight to synthesise enough vitamin d as opposed to dark skinned individuals. (Richard et al., 2017).

2.3.10 Maternal exposure to pesticides

Pesticides are one of the most well- known endocrine disruptors. A USA based population study suggests that the background exposure to pesticides leads to vitamin D deficiency. This negatively impacts calcium and phosphorus metabolism. Mothers exposed to pesticides during delivery are at high risk of vitamin D deficiency or insufficiency. Maternal vitamin D levels determine fetal levels and eventually the neonatal levels of vitamin D.(Yang et al., 2012).

2.4 The interplay between vitamin D and magnesium, calcium, parathyroid hormone and alkaline phosphatase

To maintain cellular function and the integrity of the body's structure, magnesium calcium and phosphorus are essential (Blaine et al., 2015; Favus & Goltzman, 2013; Heaney, 2011). The correct balance of minerals and bones is regulated by intricate genetic and epigenetic pathways.

2.4.1 Magnesium

Magnesium ranks fourth among abundant cations in the human body. It is a divalent positive ion that can exist both within and outside of cells. DNA and protein synthesis, excitability of neuronal tissue, cytoplasmic signalling, ATP synthesis, and the production of skeletal hydroxyapatite all require magnesium (Blaine et al., 2015). It also activates 25(OH)D to 1,25,(OH)D. In serum, it can be found both free and bound. The ECF compartment contains 1% of the body's magnesium, 85% of magnesium is located within bone, in which it is located on the surface of the apatite crystals, and the remainder is mostly found in muscular and soft tissue. Of this, half is easily exchangeable (Favus & Goltzman, 2013; San-Cristobal et al., 2010).

Magnesium levels increase when pH decreases, similar to calcium (with increased acidity). Ten per cent of magnesium is free and 60% is found in mitochondria; the magnesium is attached to ATP and other molecules intracellularly. Multiple enzymatic processes, such as those that involve ATP turnover, require the cofactor Mg2 +. Endothelin synthesis, cyclic GMP synthesis, nitric oxide (NO) synthase activity, and immunological function are all influenced by magnesium, which also affects free radicals. Magnesium inhibits the excitatory NMDA receptor reducing membrane excitability in neuronal and propulsion

tissue. It is a crucial cofactor for controlling DNA transcription and translation, glycolysis, phosphorylation, excitability and impulse conduction within nerves and muscles, and oxidative metabolism by mitochondria. It is necessary for the parathyroid chief cell to secrete PTH (but not for it to produce it).

Net intestinal magnesium uptake is unrelated to calcitriol and directly related to dietary intake (Favus & Goltzman, 2013). High phosphate intake, digestive issues, or long-term laxative usage can all decrease the intestinal absorption of magnesium. Magnesium is also eliminated through the digestive system.

2.4.2 Calcium

Calcium is mostly found in bone, although it is also contained in the cell cytoplasm and extracellular fluids. It combines with phosphate to create the bone's apatite crystals [Ca10(PO4)10(OH)2], which make up 65% of the bone's mass and are essential for supporting exoskeleton as well as serving as a calcium storage that can be quickly mobilised for cellular function. Although 99% of the calcium in the body is in an insoluble form, the 1% of calcium which is free helps control gene expression, cytoplasmic signalling pathways; rhythmicity of the heart; coagulation; muscular contraction; enzyme activity; secretion of exocrine and endocrine factors; fertilisation; and cell death and proliferation. (Cahalan, 2010).

About 45% of the total blood calcium is active physiologically as free ionised extracellular calcium (Ca2 +), while the remaining half is complexed to serum proteins, 5% is chelated to inorganic and organic anions. Serum pH, phosphate, parathyroid hormone (PTH), albumin, creatinine, and ionised calcium concentrations are all correlated.

The plasma membrane calcium-detecting receptor, parathyroid hormone and its receptor, calcitonin, and vitamin D work together to keep the serum calcium concentration within

specific ranges. The kidney, bone, and digestive tract are all affected by how this system functions.

Calcium absorption in the intestine and tubular epithelial reabsorption are both improved by vitamin D (calcitriol). Calcium is released from the apatite crystal by PTH and calcitriol, whereas PTH secretion is inhibited by calcitonin, released by the parafollicular C cells in the thyroid.

2.4.3 Phosphorus

Humans contain phosphorus in both inorganic and organic forms, both of which are complexed with sodium, calcium, or magnesium (Kinoshita & Fukumoto, 2018; Minisola et al., 2017). It is also an essential component of bone minerals, cell membranes, and signalling pathways. It is also essential for the production, and turnover of energy as ATP. The amount of phosphate consumed, absorbed in the intestines, and eliminated by the kidneys all affect serum concentrations. 25(OH)D, PTH, and fibroblast growth factor 23 all work together to keep this equilibrium. Age, sex, rate of growth, food, and blood calcium levels all affect serum concentration.

In the form of hydroxyapatite, bones store about 85% of the body's phosphate. About 14% of phosphate is found intracellularly, and the remaining 1% is found in interstitial fluid or serum.

2.4.4 Alkaline phosphatase

The phosphate group is removed from organic substrates by this member of a family of membrane glycoproteins. The skeleton, teeth, liver, and kidney all contain tissue nonspecific alkaline phosphatases as well as the alkaline phosphatases specific to tissues of the intestines, placenta, and germ cells (Whyte, 2017).

Alkaline phosphatase in bone performs the following functions: (1) complexes with type I collagen to prepare the bony matrix for mineral deposition; (2) shuttles ionised 25(OH)D and inorganic phosphate into the cell; and (3) hydrolyses phosphoethanolamine, pyrophosphate and other organic phosphates, to increase the levels of phosphate hence promoting the accumulation of calcium phosphate as hydroxyapatite.

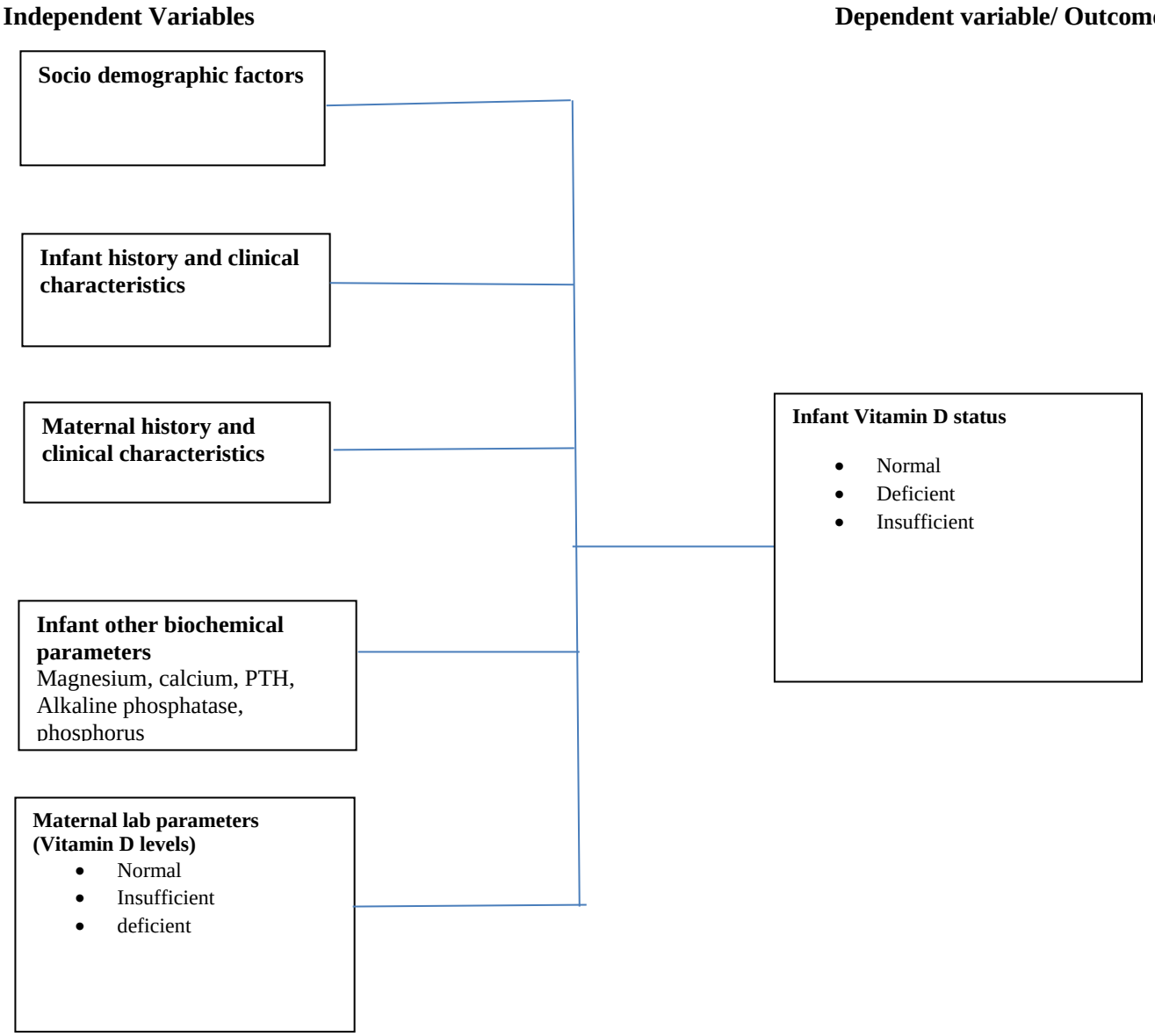
2.4.5 Parathyroid hormone

PTH boosts calcitriol production, lowers blood phosphate levels, raises plasma Ca2+ levels, and has catabolic and anabolic actions on bone tissue. Through the calcium-sensing receptor, calcium levels influence the transcription of PTH and its secretion, either enhancing (when calcium is low) or repressing (when calcium is high) these processes. Calcium levels also influence the rate of chief cellular proliferation, a reaction also facilitated by the calcium-sensing receptor.

The non-classical actions of PTH have been discovered, besides their traditional action, mineralisation. These non-classical actions include (1) its direct, quick activation of calcium uptake by the intestines that is not dependent of their influence on 25(OH)D metabolism; (2) its activation of hepatic glucose production; (3) acute urinary loss of sodium and calcium; and (4) enhancement of granulocyte migration in vitro.

Most of the non-classical physiological effects of intact PTH are not mirrored by the amino-terminal fragment PTH1-34, leading some researchers to hypothesise that they may be connected to the carboxyl end of the protein. In fact, PTH's carboxyl-terminal fragments could have biological effects that are the opposite of those of its amino-terminal section, such as hypocalcemic features that may be mediated by apoptosis-inducing effects on both osteoclasts and osteocytes(Scillitani et al., 2004).

CONCEPTUAL FRAMEWORK



STUDY JUSTIFICATION

Children's general health suffers when they are vitamin D, 25(OH)D and magnesium deficient. Low immunity, autoimmune disease onset, type 1 diabetes, and cardiovascular risk have all been linked to it. Kenya has ample sunlight for the majority of the year, but socioeconomic position, dressing style, and nutritional factors play an equally significant role in determining infants' final 25(OH)D levels.

There is a high frequency of 25(OH)D deficiency, according to a few research done in Kenya's cities. Even less research has thoroughly examined the co- existence of magnesium deficiency with VDD.

Therefore, a study on 25(OH)D levels and magnesium levels is crucial in rural areas, particularly given the rise in "hidden hunger" instances. This study may strengthen the previously compelling evidence of low 25(OH)D and Magnesium levels, even in infants who appear healthy, and make the argument for increased supplementation. Better infant growth and development, as well as the avoidance of a wide range of disorders throughout the lifespan, depend on this.

OBJECTIVES

Broad objective

To assess the vitamin D and Magnesium status among infants attending the well-baby clinic at Chuka County Referral Hospital.

Specific Objectives

1. To determine the prevalence of vitamin D deficiency among infant-mother pairs attending well baby clinic at Chuka County Referral Hospital.
2. To determine the co-existence of Vitamin D deficiency with Magnesium deficiency among infants attending well baby clinic at Chuka County Referral Hospital.
3. To determine the risk factors associated with vitamin D deficiency among infants attending well baby clinic at Chuka County Referral Hospital.

3.0 MATERIALS AND METHODS

3.1 Study design and setting

This was a cross-sectional descriptive study conducted among infants aged six weeks to one year and their mothers attending the well baby clinic at Chuka County Referral Hospital.

Chuka County Referral Hospital is a level 4 hospital that serves as the main referral hospital for Tharaka-Nithi County in Eastern Kenya. It caters for an estimated population of 200,000 people. It has an inpatient bed capacity of 150, and attends to approximately 100, 000 patients annually. The latitude Coordinate of Chuka is 0^0 19’ 59’’ S.

The clinic runs every Monday to Friday and caters for an estimated 30 infants daily. It is manned by two pediatric clinical officers and 3 dedicated nurses.

3.2 Study population

We included all infants aged 6 weeks to 1 year and their mothers attending MCH clinic. This was subject to informed consent from the mothers. We used Consecutive Sampling to recruit participants.

The following patients were excluded in the study

- Infants on treatment for rickets
- All infants on calcium and vitamin D supplements.

3.3 Sample size determination

Using the formula $n = \frac{Z^2 P (1-P)}{d^2}$ (Daniel, n.d.) 1999

Where *n* is the sample size, *Z* represents the level of confidence, which is 1.96 for a 95% confidence interval, *P* is the expected prevalence or proportion based on a previous study of a similar nature, and *d* is precision (in proportion of one; if 7.5%, d = 0.05; typically preferred in many studies).

According to a research by Urio et al, 22% of babies had vitamin D insufficiency (Urio et al., 2021). **This study was selected for calculation of sample size because their study population was similar to the population we are planning to study. It was also conducted in an African setting.**

With these values, the sample size was calculated with a P value of 0.22 and a d value of 0.05, as shown below: 0.005625

$$n = (1.96^2 \times 0.22 \times 0.78) / 0.075^2$$

$$n = 117$$

3.4 Recruitment and Consenting Procedures

In the well baby clinic at Chuka hospital, clients are registered and triaged. Upon triage they are usually directed to the consultation rooms based on the services they are seeking.

The participants were recruited after they received the primary service that brought them to the hospital. This was done to ensure no interruption of services.

Potential study participants were directed to one of the consultation rooms where they were recruited consecutively. In the recruitment room, the research team introduced themselves to the caregivers and explained the purpose of the study, the potential benefits and risks with an emphasis on confidentiality of the information that was collected.

A written informed consent was obtained from those willing to participate in the study.

Those recruited (Mother- Infant pairs) into the study were assigned a unique Study identification number. This unique study number appeared on the participant's questionnaire and laboratory sample collection vacuettainers and the printed laboratory result. No hospital patient identifiers appeared on the questionnaire or the laboratory tools. We also kept a Master register containing the participants unique number, their hospital registration number and their contact details. This master study register was crucial in tracing those found to have any biochemical derangements to offer them treatment and follow up. Those found to have normal findings, were given the positive feedback of their results by the principal investigator. The Master register was kept securely by the Principal investigator to ensure confidentiality.

Those who opted out of the study and those who consented were offered health education on Vitamin D deficiency prevention and given pamphlets explaining the same.

3.5 Study procedures

Four research assistants, 2 Clinical officers and 2 nurses were recruited and fully trained on the research purpose, procedure and ethics. They were trained on how to administer the study questionnaires, take history and examine patients; standard precautions, and techniques of blood sampling. All potential study participants and their guardians were approached on arrival at the well baby clinic by the research team who explained the study purpose, potential benefits and risks and written informed consent was obtained from those willing to participate in the study.

We identified and enlisted infants aged 6 weeks to 12 months and their mothers who were visiting the MCH clinic. To find the participants, we used consecutive sampling.

The principal researcher worked with the team of four research assistants to gather demographics, clinical history, physical characteristics and draw blood samples from study participants.

The interviews, physical examination, sample collection of the consented participants took place in one of the consultation rooms. This ensured confidentiality and privacy of the participants was upheld.

A thorough clinical history was obtained and documented in a questionnaire. Along with maternal features, we also incorporated sociodemographic traits. To find signs of bone mineral disorders as well as other anthropometric factors, a physical examination was conducted. The study variables are described in full below.

3.5.1 Sociodemographic characteristics

Data on the patient's age, sex, gestational age at birth, method of feeding and assessment of exposure to sunlight was collected using a pre-designed questionnaire. We used Bristol breastfeeding validation tool to assess for breastfeeding. We collected maternal information, including the mother's use of chronic medications, contraceptives, sunscreens, vitamin D supplementation and exposure to pesticides. Additional details gathered included the caretaker's economic situation and affordability of vitamin d supplements, type of housing (maisonette or flats), the number of children, and the spacing between births.

3.5.2 Definition of adequate sun exposure

A minimum of five days per week with at least thirty minutes of sunlight exposure to the face, back of the hands, forearm, and part of the trunk was considered enough sun exposure (Tangpricha, 2015).

To estimate the body surface area exposed to sunlight, we used the Lund browder infant chart.

In order to detect clinical rickets signs such craniotabes, rachitic rosary, widened/thickened wrists or ankles, bowlegs, and Harrison groove, we thoroughly examined each study participant.

3.5.3 Anthropometric measurements

All eligible participants in the study had their anthropometric measures taken while wearing loose clothing and bare feet.

- Both height and weight were taken. Age percentiles for height and weight were

computed.

- Measurements were made twice for each anthropometric index, and the average were determined.

3.5.4 Laboratory parameters

Three millilitres of venous blood was taken from the study participants' antecubital veins and transported to Chuka County Referral Hospital lab. The following laboratory analysis was carried out:

- The fluorescent immunoassay approach was used to assess vitamin D levels in mother-infant pairs. Serum was extracted from 3 ml of blood using a centrifuge and used in the analysis. The amount of vitamin D was expressed in ng/dl, with a sufficiency level of 30ng/ml, an insufficiency level of 20.1-29.9ng/ml, and a deficiency level of 20ng/ml.
- Magnesium, alkaline phosphatase, calcium, parathyroid hormone and phosphorus levels were measured in infants. The cutoff values for these parameters were as follows:
    - Magnesium 0.8- 1.1 mmo/l
    - Calcium 2.2-2.6 mmol/l
    - Alkaline phosphatase < 130 IU/l
    - Parathyroid hormone 10.2-55pg/ml for those with low calcium levels.
    - Phosphorus 0.87- 1.55 mmol/l
    - Albumin levels for those with low calcium levels 35- 55g/l

3.6 Biochemical determination of serum vitamin D levels

In a red vacutainer, 3 ml of venous blood was centrifuged within an hour.

The amounts of vitamin D in the samples was determined biochemically using the fluorescent immunoassay technique. The test is based on the idea that when a sample and reconstituted marker are combined, the target substance in the sample binds to the detection antibody that has been fluorescently labelled, creating a complex that is then analysed. The excess fluorescent- labeled antibody travels forward to the test line via capillary action when the sample combination is added to the sample well of the test device, where it mixes with the 25(OH) D that is immobilised by the test strip. The less fluorescent-labeled antibody accumulated in the test zone, the more target material there is in the blood sample.

The amount of vitamin D is indicated by the signal intensity of the detector antibody's fluorescence, and the FinecareTM FIA Meter displays vitamin D levels in the blood sample. In relation to the vitamin D concentration, the signal is inversely proportional. The FinecareTM FIA Meter displays XXX ng/mL as the test's default result unit.

3.7 Biochemical determination of Intact Parathyroid Hormone (iPTH) levels

The Maglumi machine utilises an immunoluminometric test. A monoclonal anti-PTH antibody was used to label ABEI, while a different monoclonal antibody was used to label FITC. A

sandwich was formed by carefully combining the sample, calibrator, or control, the anti-FITC-coated magnetic microbeads, the FITC label, the ABEI label, and the FITC label.

We decanted the supernatant following sedimentation in a magnetic field, followed by a single cycle of washing. The beginning reagents were then included, and a flash chemiluminescence reaction was then started. A photomultiplier recorded the optical pulses as RLU in three seconds, and this measurement was dependent on the amount of PTH contained in the controls or samples.

3.8 Determination of bone biochemistry parameters and albumin levels

Using a semi-automated BTS-350 analyser, serum calcium, magnesium, phosphorus, and alkaline phosphatase were measured. This system had built-in controls and measured serum biochemical levels using the colorimetric approach. The same machine was used to measure albumin levels in those with low calcium levels.

Only participants noted to have low calcium level had their albumin level checked and corrected calcium calculated using the formula:

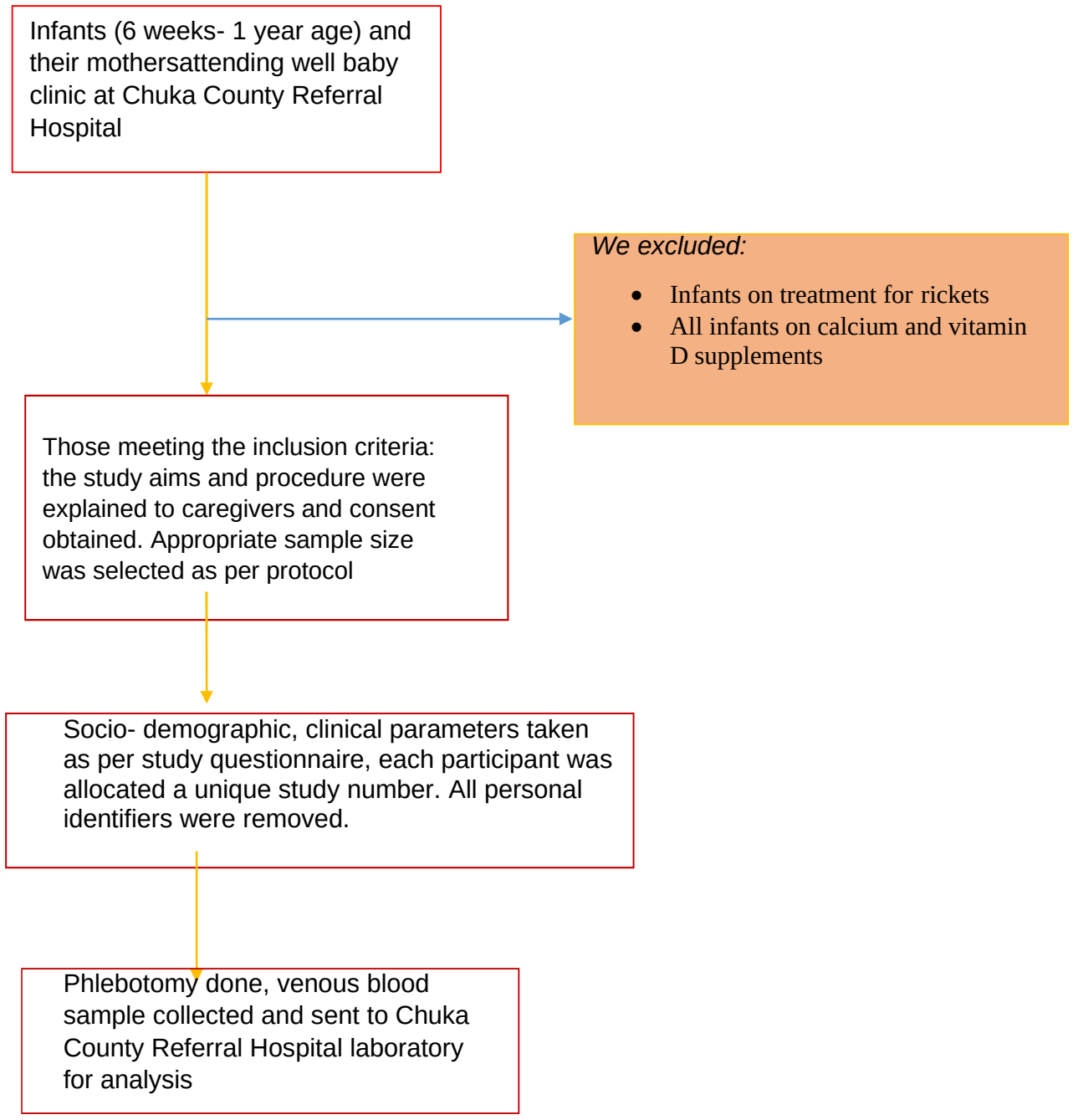
Corrected calcium mmol/l= (0.02x (normal albumin-participant's albumin) + serum calcium.

Those who had low corrected calcium levels had their parathyroid hormone levels determined too.

3.9 Quality Assurance

By instructing research assistants on the goals of the study and how to take anthropometric measurements, we ensured the accuracy of the data that is gathered. To assure quality, the principal investigator and the research assistant took these measurements twice, with the average being noted. We used calibrated height and weighing devices. Qualified staff performed laboratory measurements, calibrated equipment, and used new standards and controls. The tests had their own internal controls that complied with standard quality control of the assays used to measure vitamin D and bone biochemical composition. Every time a participant's sample was tested, this internal control was carried out. This control showed that the test instrument was properly inserted and read by the FinecareTM FIA Meter. Each fifth sample was split into two sections for external quality control, with one portion being submitted to the Lancet Pathologists' Reference Laboratory in Meru to check vitamin D levels.

STUDY FLOW CHART



Independent variables	Outcome variables
Sociodemographic variables 1. Age 2. Gender 3. Gestational age at delivery 4. Mode of feeding- Exclusive breast feeding using validating tool for breastfeeding Vs complementary feeding 5. Caregiver economic status- House wife/ employed/ self employed 6. Caregiver affordability of vitamin D supplementation 7. Residence – Flats Vs maisonette 8. Number of siblings 9. Caregiver education level and birth spacing Medical history/ clinical features 1. Duration of sun exposure and proportion of BSA exposed to sunlight during sun bathing at home 2. Proportion of BSA exposed to sunlight during clinic visit. 3. Underlying medical conditions- epilepsy, chronic kidney disease, malabsorption disorder, liver disease 4. Maternal antenatal supplementation of Vitamin D 5. Maternal use of chronic medication- antituberculous medication, steroids, ARVs and anticonvulsants 6. Maternal use of Skin bleaching agents and sunscreens 7. Any exposure to pesticides 8. Maternal use of contraceptives 9. Familial history of bone disorders- recurrent bone fractures, bone deformities 10. Clinical features of rickets Anthropometric parameters 1. Length 2. Weight 3. W/L Z score	Outcome variables for Infants 1. Serum 25 Hydroxy Vitamin D 2. Serum calcium 3. Serum magnesium 4. Alkaline phosphatase 5. Phosphorus 6. Parathyroid hormone (those with low calcium) Outcome variables for Mothers 1. Serum 25 hydroxy Vitamin D for Mothers

Statistical analysis

Data was coded, entered into SPSS version 23.0 (IBM), and cleaned. Continuous variables like age, length, weight, vitamin d and bone biochemistry levels were expressed in means ±SD, or median (Interquartile range). Categorical variables like vitamin D sufficiency, insufficiency and deficiency were analysed as proportions, n(%). Comparison and correlation of maternal versus infant vitamin D levels , vitamin D levels and magnesium levels were done using paired students't-test, with level of significance set at $p< 0.05$. Chi-squared tests were used to analyze relationships between categorical variables such as gender and vitamin D states (deficiency or insufficiency). We performed a linear regression analysis to see the relationship between Serum Maternal and Infant vitamin D levels and Infant vitamin D levels with serum Magnesium levels.

Ethical Considerations and authority

The University of Nairobi/Kenyatta National Hospital Ethics and Review Committee was contacted for ethical approval. Potential participants received a comprehensive explanation of the study in a language they could comprehend (English, Kiswahili, and in some instances, vernacular). Each Infant/ mother pair received a study number. All data was kept under strict confidentiality. Only investigators and assistants had access to the data.
All filled questionnaires were kept under lock and key and were only accessed by the principal investigator.

We made it obvious to the parents of the infants that participation in the study was entirely optional and that there were no negative consequences for those who opted out. Furthermore, participants did not receive any financial rewards**.** Those found to have deranged parameters were treated and this cost was catered by the principal investigator. All participants received health education and a pamphlet on importance of vitamin D.

We used aseptic technique to extract 3ml of blood from the participant's (mother and infant pairs) antecubital vein. The caregivers were not responsible for any fees associated with this research. Before analysis, the serum samples were kept in the Chuka County Referral Hospital's laboratory department. After three months, the samples were disposed off via incineration.

4.0 RESULTS

4.1 Descriptive statistics

Study participants

There was a total of 122 participants in the study. 56% (68) of the participants were male while 44% (54) were female. The average age of the participants was 5.5 months with the youngest participant being 1 month two weeks while the oldest was 12 months. Out of the 122 participants, only 2 were born preterm. 60% (73) of the participants were born through spontaneous vaginal delivery (SVD) while 40% (49) were born through caesarian section.

58% (71) of the participants reported sun exposure for more than 30 minutes, 40% (49) for less than 30 minutes and 2% (2) were never exposed to sunlight (figure 1). The average body surface area of infant exposure to sunlight while at the clinic was 10% and 45.3% at home.

The initial mode of feeding for participants was breastfeeding in 119 of the infants, complementary feeding in 1 infant, and formula feeding in 2 infants. At the time of the study, 64% (78) of the infants were on exclusive breastfeeding , 34.4% (42) complementary feeding, and 1.6% (2) fed using formula (figure 2). All the participants scored 8/8 in the Bristol breastfeeding validation tool.

87.7% (107) of the caregivers were from low-income households while 12.3% (15) were from middle income households. 45% (55) of the caregivers had completed tertiary education, 49% (60) secondary education and 6% (7) primary education. 55.7% of the participants' caregivers lived in apartments, 43.4% in bungalows and 0.8% in maisonettes (table 1). 70% of the caregivers reported they could afford to purchase Vitamin D supplements for their infants.

In regards to infant Weight for Length, the average length for age percentile was 44.51, average weight for length percentile 50.4 and average weight for age percentile 47.57. Their frequencies are described in table 2 (table 2).

During pregnancy, 95% of the participants' caregivers denied maternal use of medication, 97% denied the use of lightening cream and bleaching agents, 97% denied sunscreen use and 95% denied exposure to pesticides. 64% of the caregivers had previously used contraceptives. Out of the 122 participants, only 4 reported a family history of bone disorders.

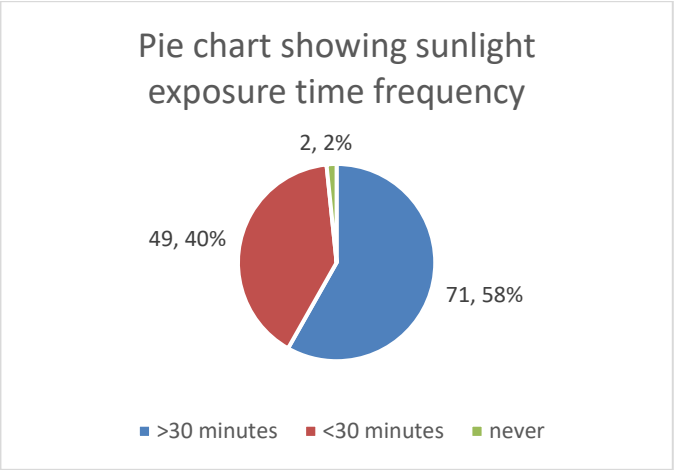


Figure 1: pie chart showing infant sunlight exposure frequency

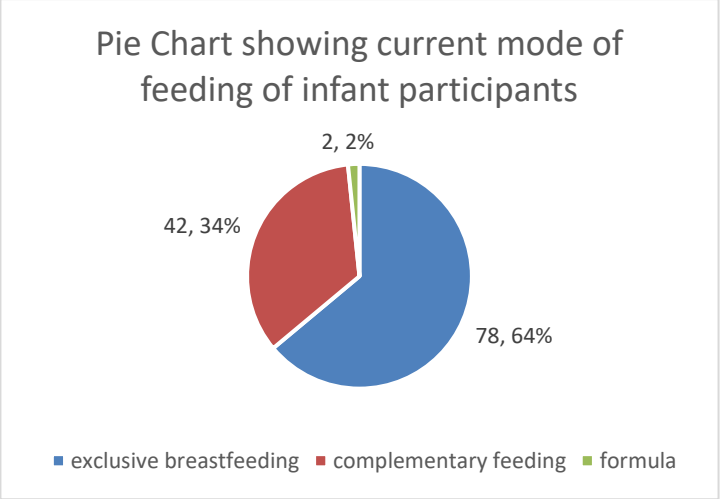


Figure 2: pie chart showing current mode of feeding of infant participants

Table 1: table showing socioeconomic characteristics of participants' caregivers

	Frequency (n, %)
Socioeconomic status	
Low-income household	107 (87.7%)
Middle income household	15 (12.3%)
Education level	
Tertiary education	55 (45%)
Secondary education	60 (49%)
Primary education	7 (6%)
Housing	
Apartments	68 (55.7%)
Bungalows	53 (43.4%)
Maisonettes	1 (0.8%)

Table 2: Table showing the frequency of infant arthropometric measurements in each category on each scale

Wt for Ht percentile	**Length for age**	**Weight for length**	**Weight for age**
Below 3	11 (7.9%)	14 (10%)	16 (11.4%)
3-25	35 (25%)	24 (17%)	22 (15.7%)
25-95	61 (43.6)	71 (50.7%)	76 (54.3%)
Above 96	13 (9.3)	11 (7.9%)	8 (5.7%)

4.2 Biochemical parameters

The average level of serum Vitamin D in the infants was 52.57 ng/dl with 5% of the participants being Vitamin D deficient. The average maternal level of serum Vitamin D was 37.95 ng/dl with 9% of the caregivers being Vitamin D deficient. The average serum magnesium levels in the participating infants was 1.8 mmol/l, average serum

calcium levels 2.41 mmol/l, average phosphorus levels 1.36 mmol/l and average levels of Alkaline Phosphate 314.5 u/l (table 2). The average levels of Parathyroid Hormone was 62pg/ml

Table 3: average maternal and infant serum levels of Vitamin D

Biochemical parameter	**Average**
Infant Vitamin D (ng/dl)	52.57
Maternal Vitamin D (ng/dl)	37.95

Table 4: average levels of serum biochemical markers

Biochemical parameter	**Average**
Magnesium (mmol/l)	0.96
Calcium (mmol/l)	2.41
Phosphorus (mmol/l)	1.36
Alkaline Phosphate (u/l)	314.5
Pararhyroid Hormone (pg/l)	62

Table 5 :showing frequency of Maternal and Infant serum vitamin D levels

Serum 25 hydroxyl vitamin D categories (ng/ml)	**Infant N (%)**	**Maternal N (%)**
≥ 30 (Normal levels)	**108 (88.5)**	**84 (69)**
20.1- 29.9 (Insufficient levels)	**8 (6.5)**	**27 (22)**
<20 (Deficient)	**6 (5%)**	**11 (9)**

Table 6: Table showing frequencies of infant serum mineral levels (mmol/l) in each category (low, medium, high)

	Normal	Low	High	Mean
Magnesium (0.8-1.1 Mmols/ L)	121 (99.8%)	0	1 (0.8%)	1.08
Calcium (2.2-2.6 Mmol/ L)	106 (86.8%)	7 (5.7%)	8 (6.6%)	2.41
Phosphorus (0.87-1.55 Mmol/l)	108 (88.5%)	0	14 (11.5%)	1.36
Alkaline Phosphate (151-471U/L)	108 (88.5)	5 (4.1%)	9 (7.4%)	314.5
Parathyroid Hormone (10.2-55pg/ml)	0	0	7 (100%)	62

4.3 Serum vitamin D and serum magnesium levels

The Kruskal Wallis test was used to compare serum magnesium levels between the three categories of serum Vitamin D levels (sufficient, insufficient, deficient) and it was seen that there was no statistically significant difference between the serum magnesium levels in all 3 groups (p=0.183).

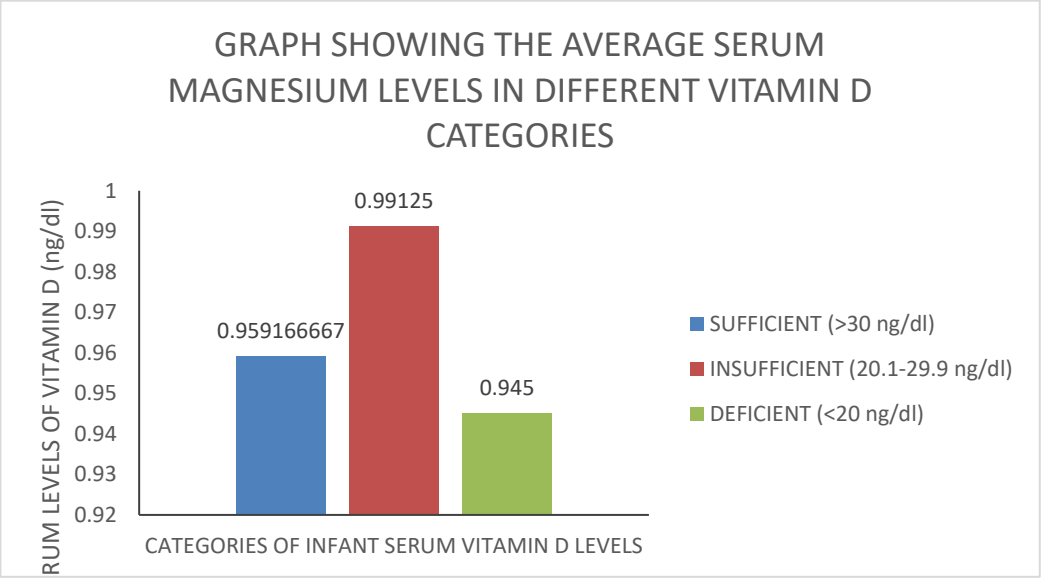


Figure 3: Graph showing the average serum vitamin D levels in different categories

4.4 Serum calcium and magnesium levels

The spearman correlation test was used to compare serum Magnesium levels with serum calcium levels. There was a positive linear correlation seen between the two biochemical parameters that was statistically significant (p=0.020). In addition, the Kruskal Wallis test was used to compare the levels of magnesium in each category of serum calcium and it was seen that there was no statistically significant difference in serum magnesium levels between groups (p= 0.282).

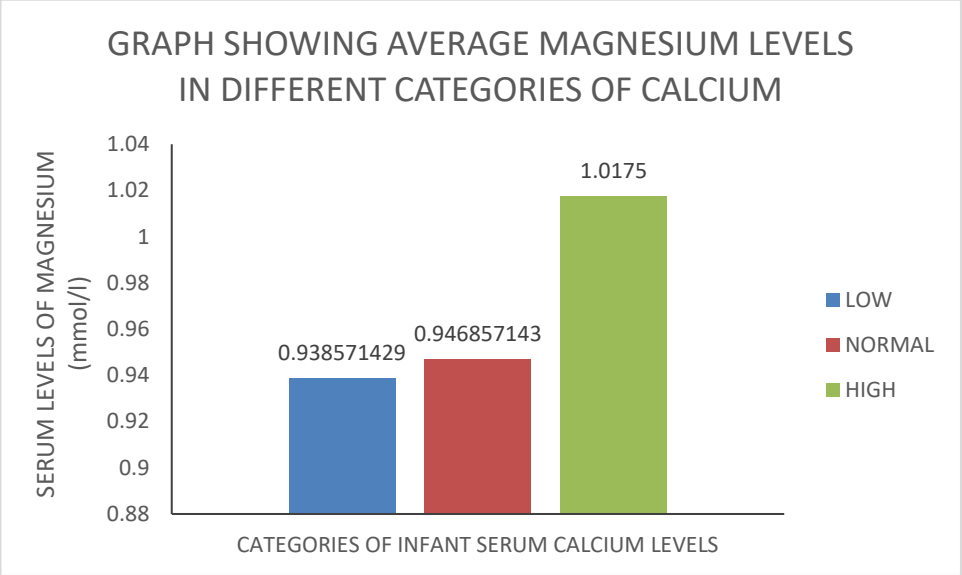


Figure 4: Graph showing average magnesium levels in different categories of calcium

4.5 Serum Vitamin D and infant BMI parameters

The Kruskal wallis test was used to compare the serum Vitamin D levels in each category of the infant BMI parameters that is: length for age, weight for length, weight for age. It was determined that there was no statistically significant difference in serum vitamin D levels in each category for each parameter (p= 0.378, 0.375, 0.558 respectively). In addition, there was no statistically significant correlation between serum Vitamin D levels and infant BMI (p= 0.095, 0.483, 0.31 respectively). (table 9)

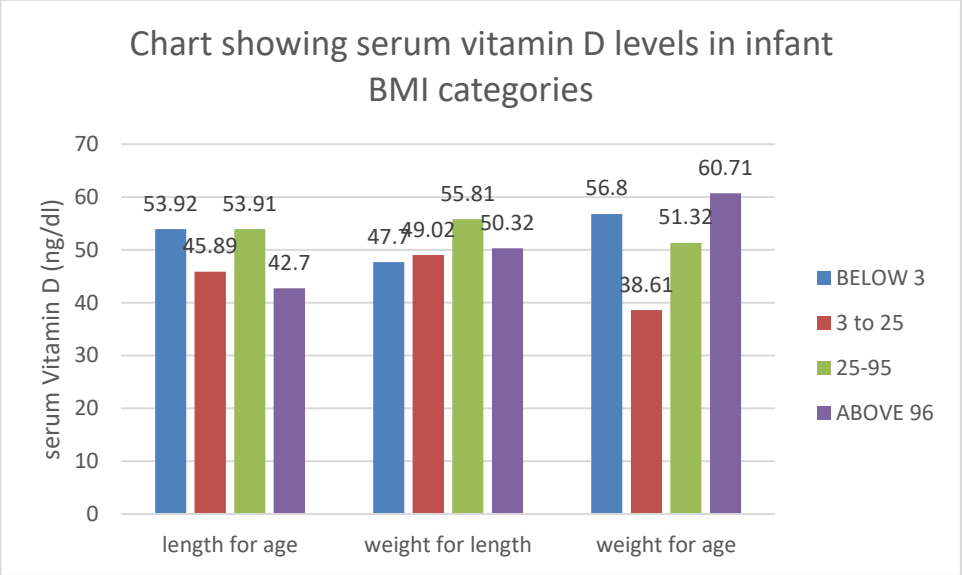


Figure 5: chart showing serum vitamin D levels in infant BMI categories

4.6 Serum Vitamin D and risk factors

Various risk factors were analyzed for association with Vitamin D status. These were biological, environmental and socioeconomic risk factors. Biological risk factors included maternal exposure to medication, gestational age at birth, mode of delivery, initial and current feeding, maternal use of contraceptives and family history. Environmental risk factors included maternal use of sunscreen and skin bleaching/ lightening cream, infant exposure to sunlight and maternal exposure to pesticides. Socioeconomic risk factors included economic status, housing status and education level. A binary logistic regression test was conducted to identify the predictors of Vitamin D deficiency among the infant participants. It was determined that there was no statistically significant predictor of suboptimal (10-30 ng/dl) vitamin D levels in the risk factors assessed. The results are shown in the table below. (table 10).

Table 7: predictors of suboptimal and optimal vitamin D status among infant participants

		Neonatal vitamin D level frequency (n)		P value	OR	95% CI for	
		suboptimal	optimal			Lower	Upper
Maternal medication	No	14	102		Ref		
	Yes	0	6	0.999	0.000	0.00	
Gestation age	Term	14	106		Ref		
	Preterm	0	2	0.999	0.000	0.00	
Delivery mode	Spontaneous vaginal delivery	10	63		Ref		
	Cesarean section	4	45	0.410	0.572	0.152	2.162
Initial Feeding mode	Formula/ complimentary	14	107		Ref		
	Exclusive feeding	0	1	0.490	2.641	0.168	41.632
Current feeding mode	Formula/ complimentary	8	36		Ref		
	Exclusive			0.428	1.713	0.452	6.498
Contraceptive use	Yes	8	70		Ref		
	No	6	36	0.608	1.390	0.395	4.889
Family history	No	13	105		Ref		
	Yes	1	3	0.550	2.295	0.15	35.05
Sunscreen use	No	13	106		Ref		
	Yes	1	2	0.542	2.388	0.145	39.271
Bleaching/ lightening cream	No	14	104		Ref		
	Yes	0	4	0.999	0.000	0.00	
Duration of sunlight exposure	More than 30 minutes	8	63	0.656	Ref		
	Less than 30 minutes	6	43	1.0	0.275	0.00	
	Never	0	2	1.0	0.714	0.00	
Clinic surface area of exposure		-		0.467	1.053	0.924	1.200
Home surface area of exposure		-		0.862	1.004	0.961	1.049
Maternal exposure to pesticides	Yes	14	102		Ref		
	No	0	6	0.999	0.000	0.00	
Economic status	Middle income	2	13		Ref		
	Low income	12	95	0.722	1.417	0.207	9.692
Housing	Maissionettes	0	1	0.941	Ref		
	Bungalow	6	47	1.00	0.00	0.00	
	Block of flats	8	60	0.728	1.357	0.243	7.578
Education level	Tertiary	14	100	0.553	Ref		
	Primary	0	8	0.212	0.463	0.138	1.553
Affording vitamin D supplements	Yes	12	73		Ref		
	No	2	35	0.190	4.116	0.497	34.111

4.7 Infant and maternal serum Vitamin D

The maternal serum Vitamin D levels in each category of infant Vitamin D status were compared using and it was determined that there was statistical significance (p=0.001). In addition, spearman correlation analysis was conducted and it was determined that infant and maternal serum vitamin D levels. Linear regression tests revealed a positive correlation between serum infant and maternal vitamin D levels (r= 0.285, p= 0.002) (table 11).

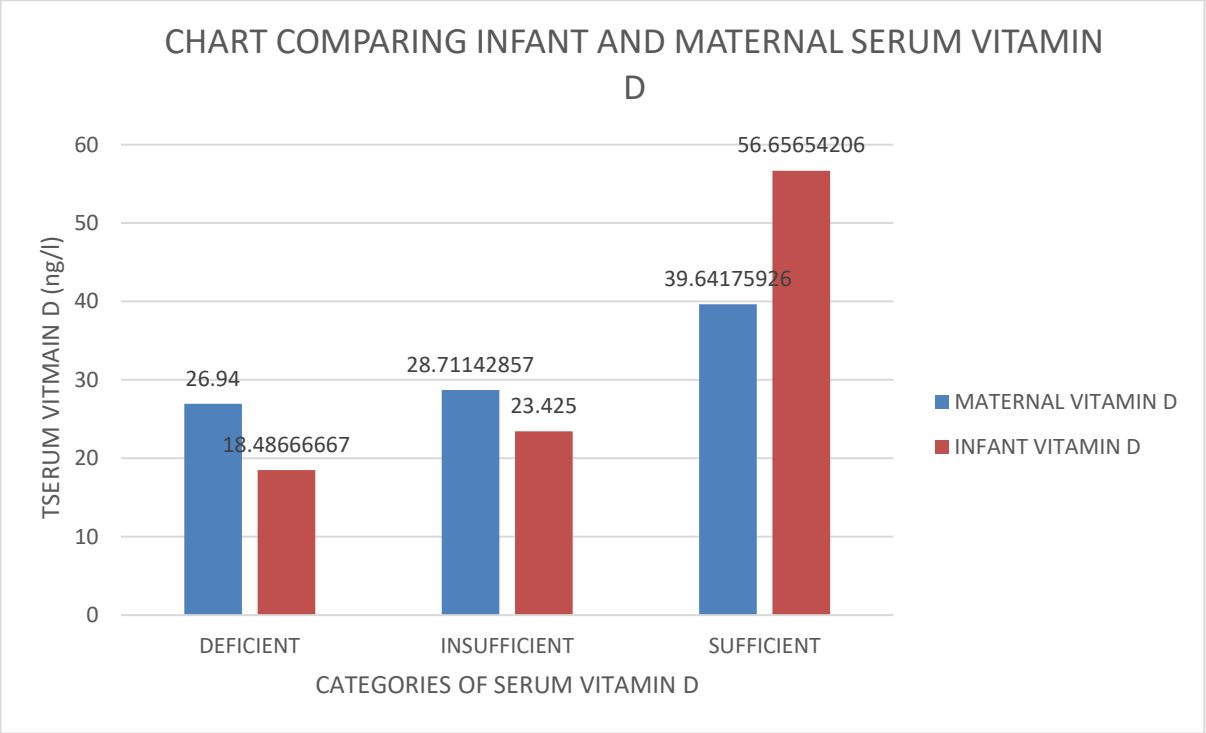


Figure 6: chart comparing infant and maternal serum Vitamin D

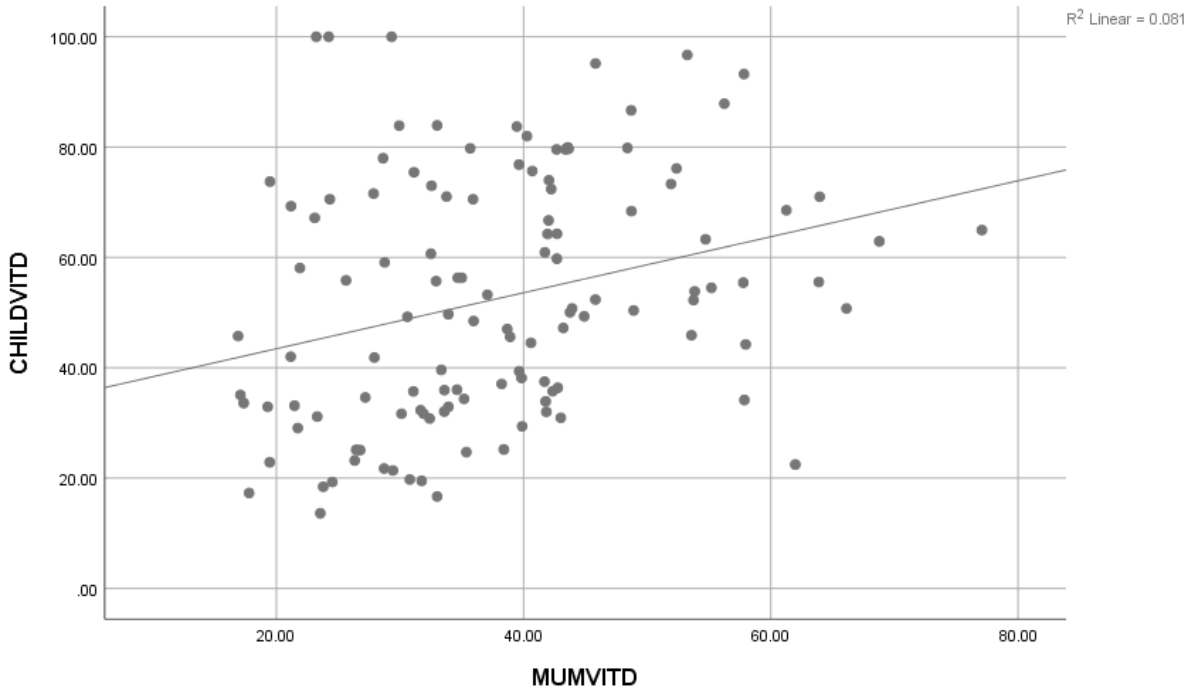


Figure 7: chart showing relationship between infant and maternal vitamin D levels

4.8 Serum vitamin D levels across different age groups

The serum vitamin D levels were compared among infants aged 6 months and below and those older than 6 months. Those below 6 months of age had an average serum Vitamin D level of 53.296 ng/dl while those above 6 months had an average serum Vitamin D level of 53.122 ng/dl. This difference was not statistically significant (p= 0.839) (table 13). Binary logistic regression determined a 0.43% predictor value of suboptimal vitamin D levels (10-30 ng/dl) in infants under 6 months of age, as compared to those over 6 months, OR= 0.434% 95% CI (0.001, 20.186). (Table 14).

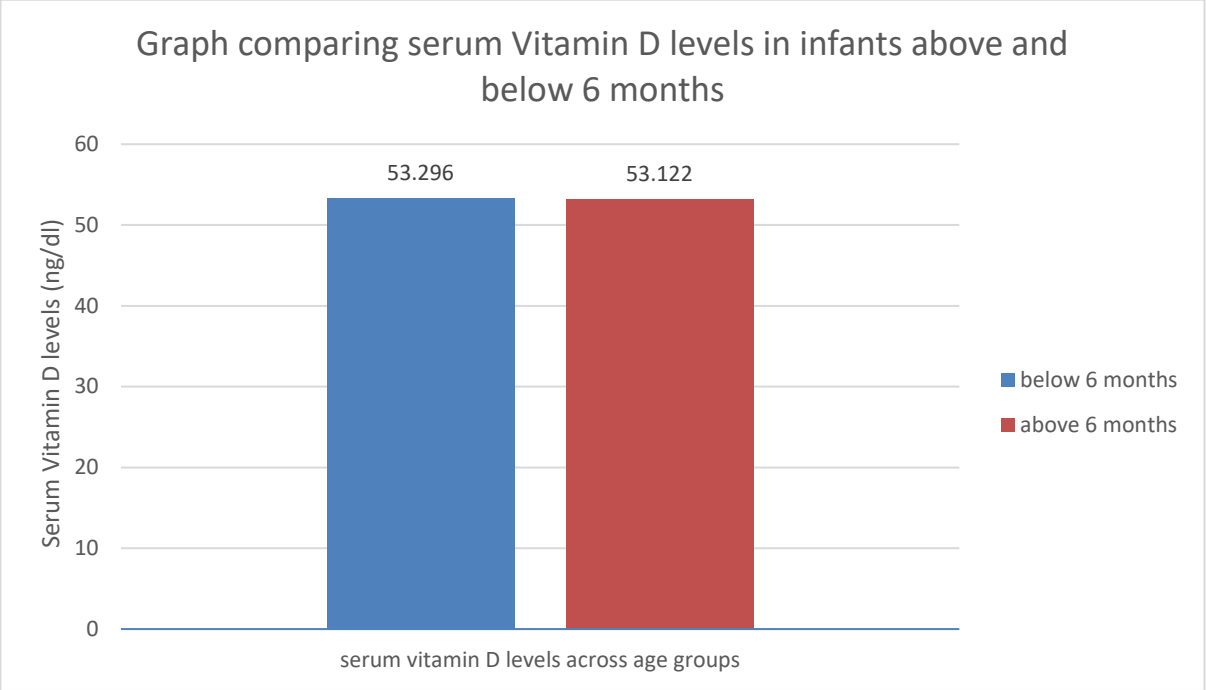


Figure 8: graph comparing serum Vitamin D levels in infants above and below 6 months

Table 8: table showing results of binary logistic regression showing age as a predictor of suboptimal vitamin D levels

Variable	**P value**	**OR**	**CI 95%**
Below 6 months	0.434	7.340	0.001, 20.186

5.0 DISCUSSION

5.1 Prevalence of Vitamin D deficiency

Our study population involved 122 infants aged 6 weeks to 12 months and their mothers. The average age of the infants was 5.5 months. Majority of the infants were born at term via spontaneous vaginal delivery and were on exclusive breastfeeding. 58 % of them had sun exposure for more than 30 minutes.

In this study, we found the prevalence of vitamin D deficiency and insufficiency among infants to be 5% and 6.5% and that among Mothers to be slightly higher at 9% and 22% respectively. The total hypovitaminosis D was 11.5% and 31% %. This is lower than what has been reported locally, regionally and internationally. A study done in the Kenyan capital among exclusively breastfed infants showed overall hypovitaminosis D of 33.4% (Said et al., 2020) . Another study done in rural Western Kenya showed high infant and Maternal vitamin D deficiency at 30 and 21% respectively (Toko et al., 2016). Chacham et al found a prevalence of 74 % in India while in Germany the prevalence was 94% and 35% in winter and summer respectively(Chacham et al., 2020; Wuertz et al., 2013). Some of the differences observed in Vitamin D levels across different populations can be attributed to the geographical location and mean age of the infants. For instance the study by Wuertz was done in a country with colder climate and a higher latitude. Our study was done in a region that is very near the equator and most of the study population reported daily sun exposure of more than 30 minutes. Majority of Mother infant pair studies on

Vitamin D levels across the globe have focused on infants during neonatal period as opposed to our study that looked at older infants. Such infants have had exposure to the environment and this could explain the lower vitamin D deficiency rate in our study. Other factors could be the study design and the cut off values for vitamin D adopted by the authors. The Western Kenya study adopted <50nmol/l and <75nmol/l as deficient and insufficient respectively(Toko et al., 2016).

5.2 Correlation between vitamin D deficiency and magnesium levels among infants

We found a mean serum magnesium of 1.8mmol/l. Our study did not find any case of hypomagnesemia. This is consistent with values recorded in Mexico and USA. (Murphy et al., 1992). When compared among infants with vitamin D sufficiency, insufficiency and deficiency, there was no statistically significant correlation between magnesium and vitamin D levels. There was a positive correlation between serum calcium and magnesium levels.

Our findings may be partly explained by local weaning practices; most infants are weaned on foods rich in magnesium such as bananas, green leafy vegetables, cereals and legumes . Most of the infants were on exclusive breastfeeding. Median levels of magnesium in breastmilk of healthy lactating women is approximately 1.27- 1.4 mmo/l with a range of 0.62- 2.63 mmol/l and remains fairly stable throughout lactation period (De Baaij et al., 2015). Further, the low prevalence of vitamin D deficiency in this population means that magnesium levels are likely to be normal.

Approximately 65% of the total body magnesium is found within bones. Magnesium plays a key role in magnesium-dependent adenyl-cyclase, which is involved in the release of parathyroid hormone and activity of bone. It also has a major role in activation of Vitamin D. All enzymes that metabolise vitamin D require magnesium which acts as a co factor in the enzymatic reactions in the liver and the kidneys (Uwitonze & Razzaque, 2018). Due to this link, a deficiency of magnesium would cause deficient parathyroid hormone release and peripheral resistance to parathyroid hormone, low levels of activated vitamin D with subsequent hypocalcemia.

Due to this link between parathyroid hormone, vitamin D, calcium and magnesium, it is imperative that infants with vitamin D deficiency be screened for magnesium deficiency, and repletion be done when it is low(Dandinavar et al., 2019; Favus & Goltzman, 2013; Khundmiri et al., 2016).

5.3 Factors associated with Vitamin D deficiency in the infants

In this study, the main predictor for infant 25 hydroxyvitamin D deficiency was low maternal vitamin D. Infants whose mothers had low vitamin D levels were more likely to have hypovitaminosis D. This mirrors the findings by Atandi et al that revealed that mothers who had low vitamin D levels were more likely to deliver neonates who were vitamin D deficient (Atandi, 2018). There is evidence that vitamin D levels of breastfeeding mothers correlates with that of their infants. Babatunde et al found a positive correlation between paired maternal and breast feeding mothers (Babatunde et al., 2018). In our study, this correlation could be attributed to the fact that mothers who have low vitamin D levels are more likely to stay indoors with their infants and their nutritional sources of vitamin D are similar to those of their infants on complementary feeding. There is further evidence that maternal vitamin D correlates with those of their breastfeeding infants but this correlations seems to fade from 4 to 6 months postnatal age(Merewood et al., 2010; Suaini et al., 2014).

Most studies have reported exclusive breast feeding as one of the predictors of low vitamin D levels in the infants. However, our present study did not find any correlation among those who were exclusively breastfed versus those who were on complementary feeding. A south African study reported a higher incidence of Vitamin D deficiency among infants who were on exclusive breastfeeding(Ncayiyana et al., 2021) . Other risk factors that have been reported in various studies though were negative in our study include age less than 6 months, dark skin, poor sun exposure, low socioeconomic status and infants born in winter (Chacham et al., 2020; Gordon et al., 2008).

6.0 CONCLUSION AND RECOMMENDATIONS

In this study,

- The prevalence of hypovitaminosis D was 11.5% and 31% among infants and mothers respectively.
- There was no co-existence of Vitamin D deficiency with magnesium deficiency.
- The risk factor for low vitamin D levels among infants was low maternal vitamin D levels.

RECOMMENDATIONS

- We recommend a study examining seasonal changes in vitamin D status to compare levels during sunny seasons and cold seasons of the year.
- Despite the relatively low prevalence of vitamin D deficiency, we recommend screening of breastfeeding mothers for vitamin D deficiency
- Policies on sun exposure, diet rich in vitamin D and possible routine supplementation of vitamin D for mothers and their infants.

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APPENDICES

APPENDIX 1: DATA COLLECTION TOOL

Study Title: **VITAMIN D AND MAGNESIUM STATUS AMONG HEALTHY INFANTS AGED 6 WEEKS TO 12 MONTHS ATTENDING CHUKA COUNTY REFERRAL HOSPITAL WELL BABY CLINIC.**

Study unique identification No:_____

Sociodemographic information

1. Study ID number_____
2. Age (Months)_____
3. Gender Male ☐ Female ☐ (Tick one)
4. Gestational age at delivery: Term ☐ Preterm ☐

   (term is 37 completed weeks)
5. Mode of delivery SVD ☐ Caesarean section ☐ Breech ☐
6. Birth Order
   a) 1st born ☐
   b) 2nd born ☐

c) >3rd Order ☐

Past/current medical History

7. Duration of sun exposure

   a) Never ☐

   b) less than 30 minutes ☐

   c) more than 30 minutes ☐

8. Body surface area exposed to sunlight in percentage (%)

   a) During clinic visit

   b) At home (as reported by the mother)

9. Mode of feeding during 1st 6 months (for those older than 6 months)

   a) Exclusive breast feeding ☐

   b) Formula ☐

   c) Complementary feeding ☐

10. Current mode of feeding

   a) Exclusive breast feeding ☐

   b) Formula ☐

c) Complementary feeding ☐

11. If breastfeeding, is the technique appropriate (use the Bristol Breastfeeding assessment tool as provided)

a) Good technique (a score of 8/8)

b) Poor technique/ needs improvement (score =/< 7)

(If poor technique, teach mother on the correct technique as highlighted in the bristol tool)

12. Can caregiver afford to buy Vitamin D supplementation at current Market price of around 4 dollars (ksh 400-500)?

a) Yes ☐

b) No ☐

13. Caregiver Education level

a) Basic (primary school) ☐

b) Secondary ☐

c) Tertiary ☐

14. Maternal History of Medication Use (anti-tuberculous medication, anticonvulsants, antiretroviral drugs or steroids.)

a) Yes ☐

b) No ☐

15. Maternal use of skin lightening creams or bleaching agents

a) Yes ☐

b) No ☐

16. Maternal use of sunscreen

a) Yes ☐

b) No ☐

17. Maternal use of contraceptives

a) Yes ☐

b) No ☐

18. Maternal exposure to pesticides

a) Yes ☐

b) No ☐

19. Family history of bone disorders like deformed limbs, relatives with recurrent fractures,

c) Yes ☐

d) No ☐

20. Caregiver Economic status

(acoording to KNBS 2017)

a) Low income (<23,670) ☐

b) Middle income ((23671- 119,999) ☐

c) High income (>120,000) ☐

21. Residential housing

a) Block of flats ☐

b) Massionette ☐

Anthropometric measurements

22. Length (cm) _

23. Weight (kg) _

24. length for age percentile

a) below 3rd

b) 3-25

c) 25-95

d) > 97

25. Weight for length

a) below 3rd

b) 3-25

c) 25-95

d) > 97

26. Weight for age percentile

a) below 3rd

b) 3-25

c) 25-95

d) > 97

27. Clinical signs of rickets

a) Widened wrists

b) Rachitic rosary

c) Bowing of legs

d) Craniotabes

Laboratory parameters

28. Vitamin D levels of the infant (ng/dl)

29. Category of vitamin D status

≥30ng/dl (sufficiency) ☐

20.1-29.9ng/dl (insufficiency) ☐

<20ng/dl (deficiency) ☐

30. Vitamin D levels of the mother (ng/dl) _

31. Category of vitamin D status in the mother

≥30ng/dl (sufficiency) ☐

20.1-29.9ng/dl (insufficiency) ☐

<20ng/dl (deficiency) ☐

32. Magnesium levels of the infant

33. Category of Magnesium levels (use reference values 0.8-1.1 mmol/l)

Normal------) ☐

High------) ☐

Low ------) ☐

34. Parathyroid Hormone levels of the infant (only those with low corrected calcium)

35. Category of Parathyroid Hormone levels (use reference values 10-55pg/l)

Normal------) ☐

High------) ☐

Low ------) ☐

36. Calcium levels of the infant

37. Category of calcium levels (use reference values 2.2- 2.6 mmol/L)

Normal ------) ☐

High ------) ☐

Low------) ☐

38. If calcium low, check albumin level (use reference values 35-55g/l)

Albumin level _____

If albumin low (< 35g/l) calculate the corrected calcium level using formula 0.8 x (normal albumin- patient's albumin) + serum calcium

39. Phosphate levels of the infant _

40. Category of phosphate levels(use reference values 0.87- 1.55 mmol/L)

Normal ------) ☐

Low------) ☐

High -----) ☐

41. Alkaline phosphatase (ALP) levels of the infant _

42. Category of ALP levels (use reference <130)

Normal ------) ☐

High -----) ☐

APPENDIX II: INFORMED CONSENT FORM (ENGLISH)

Study Title: **VITAMIN D STATUS AND MAGNESIUM AMONG HEALTHY INFANTS AGED 6 WEEKS TO 12 MONTHS ATTENDING CHUKA COUNTY REFERRAL HOSPITAL WELL BABY CLINIC.**

Study number:

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Purpose and Benefits

We wish to conduct a research on infants attending the well baby clinic at Chuka County Referral Hospital, to determine their vitamin D and bone biochemistry status. We will include infants aged 6 weeks to 12 months. We shall also draw your (mother's) blood sample to determine your blood levels of vitamin D.

If you or your infant is found to have deficiency of vitamin D or deranged biochemical parameters, or both, we shall give you nutritional advise and prescribe the relevant supplements for you and your infant. The cost of treatment will be catered by the researcher. We shall also link the infants to care at Paediatric Outpatient Clinic for consistent follow up until the parameters normalise. Each one of you will receive a pamphlet on importance of vitamin D.

Procedures

This study will be conducted through a questionnaire administered with the assistance of a trained study assistant. Physical examination of your baby will be perfomed by one of the research team members qualified as a paediatrician or paediatric clinician. It will be done in one of the consultation rooms whereby privacy will be adhered to. This will be done to ascertain the infant's anthropometric measurements and to look for any features of rickets. 3 mls of your baby's venous blood will be drawn from a peripheral vein using an aseptic technique. We shall also draw 3 mls from your peripheral line aseptically to determine your vitamin D levels.

Safeguarding Privacy

The interviewer has signed a pledge to keep all information about your infant secure. His or her name will be removed from all records involved in the study. A number will be assigned to the

survey questionnaire instead. Only project staff will have access to the study data. We will not use any name when we report results of the study.

Samples disposal

After three months, we will dispose of the samples (by this time, the study period will have elapsed). There will be safe disposal as well as incineration using the Chuka Hospital incinerator**.**

Your taking part in this study will help us determine how prevalent vitamin D and other bone biochemistry deficiencies are among well babies. This will help advance knowledge on possible future interventions that might benefit these infants, e.g. supplementation vitamin D and consistent sun exposure.

There are no direct benefits to the participants. There will be little discomfort while drawing blood. However, the overall impact for all infants may be great because new data on vitamin D and other bone biochemistry parameters' deficiency will help us know the prevalence of deficiency, and form a basis for future research on whether supplementation may improve normal growth of these babies.

For those found to have any deficiency, supplementation will be offered by the principal investigator free of charge.

Risks

A blood draw does carry a risk to the participant. The risk is however minimal if carried out correctly. There will be little discomfort during the procedure of drawing the blood. We will do all possible to ensure that we minimize the discomfort. Should there be a lot of pain or discomfort that requires medical attention, or any complication, we undertake to provide the necessary care free of charge.

If you have any questions about this research, you may call Dr. Winnie Mueni Saumu on 0723317632. If you have any questions on your rights as a research participant you can contact KNH/ UoN- ERC: P.O BOX 20723- 00202, Tel. 726300-9, ext. 44355 or 44102 Nairobi or write an email to uonknherc@uonbi.ac.ke

CONSENT CERTIFICATE

Respondent Agreement

The Study has been explained to me. ***I consent to participate together with my infant in this study***. I have had a chance for my questions to be answered. I know that I may refuse to participate or stop the interview at any time without any loss of health care benefits that we are otherwise receiving. I understand that if I have questions about this study, I may contact Dr Winnie Mueni Saumu on 0721484624. Further, I understand that the information recorded by the investigator will be confidential.

Respondent Signature_____ Date_____

Interviewer Signature_____Date_____

Contacts of the investigator

Dr Winnie Mueni Saumu

The University of Nairobi

Email; saumubundi@gmail.com , Phone: 0723317632

Lead Supervisor:

Dr. Lucy Mungai

The University of Nairobi, P.O. Box 30197-00100

Email: dr.lmungai@gmail.com , Phone: 0724654135

Kenyatta National Hospital/University of Nairobi Ethics & Review Committee contacts: **P.O box 20723-00202,**

Tel. 726300, ext. 44102, 44355

Email: uonknh_erc@uonbi.ac.ke

APPENDIX III: INFORMED CONSENT FORM SWAHILI)

CONSENT FORM IN SWAHILI

FOMU INAYOELEZA IDHINI

UTANGULIZI

Mimi ni Dkt. Winnie Mueni Saumu, kutoka Chuo Kikuu cha Nairobi. Ninafanya uchunguzi kuhusu viwango vya chembe chembe ya Vitamini D na madini ya mifupa katika watoto wa chini ya mwaka mmoja.

Lengo kuu yautafiti- Lengo la utafiti huu ni kuamua iwapo watoto hawa wana viwango pungufu vya vitamini D na hayo madini mengine. Hii ni sababu upungufu huu waweza kuathiri afya yao. Pia tutaangalia kiwango chako cha madini ya vitamin D.

Taratibu zitakazohusishwa - Lazima kukubali kushiriki katika utafiti sisi kuuliza maswali machahe kulingana na karatasi ya utafiti**.** Tutapima watoto uzito na urefu wa mwili na pia tutawaangalia dalili za ukosefu wa vitamin D. Watoto watapimwa na daktari wa watoto katika chumba cha daktari. Tutawapima kuangalia dalili za ugonjwa wa matege. Tutatoa damu mililita tatu kutoka mtoto kwa utaratibu na bila majeraha ili kufanya utafiti wa viwango vya vitamini D na madini ya mifupa. Kadhalika, tutakutoa damu mililita tatu ndiposa tukupime kiwango cha madini ya vitamin D.

Baada ya miezi tatu, damu na vifaa vilivyotumika kuhifadhi damu yako na ya mtoto wako vitatupwa. Tutatumia tanuri la kuchomea takataka katika kituo cha afya cha Chuka.

Wewe ama mtoto mkipatikana na viwango duni vya Vitamini D na hayo madini, tutakueleza jinsi ya kutumia

vyakula na madawa kutibu shida hiyo. Madawa haya yatanunuliwa na mkuu wa uchunguzi. Pia utaelekezwa kliniki au hospitali kuendelea na matibabu haya.

Haki yako kama mshiriki katika utafiti huu- Ushiriki wako na mtoto wako katika utafiti huu ni kwa hiari yako.

Hata ukichagua kushiriki au ukatae kushiriki haitaathiri matibabu ya mtoto wako. Una uhuru wa kujiondoa katika utafiti huu wakati wowote. Una uhuru wa kuuliza maswali kabla ya kutia sahihi katika fomu ya idhini na wakati wa utafiti. Maswala yote yatahifadhiwa kwa siri wakati wote.

Hasara za ushiriki – Kutolewa damu kunaweza kuwa na madhara. Kwa mfano, wewe au mtoto wako mnaweza kuhisi uchungu kidogo wakati wa kutoa damu, lakini hakuna uwezekano wa madhara kubwa sana. Ikiwa wewe au mtoto atahisi uchungu mwingi, tutagharamia dawa ya maumivu.

Manufaa ya kushiriki- Mwishoni mwa utafiti huu, nitawasilisha matokeo ya utafiti katika Chuo Kikuu cha Nairobi. Habari zozote muhimu zitakazotokana na utafiti na ambazo zitafanya tiba kuwa bora, wagonjwa watafahamishwa ili hatua mwafaka ichukuliwe.

Siri- Habari zote zitakazokusanywa wakati wa utafiti zitahifadhiwa kwa siri. Ni watafiti pekee ndio wanaoweza kufikia habari za kibinafsi. Habari zitakazokusanywa zitaandikwa na kuainishwa bila kutaja washiriki.

Ikiwa una swali lolote wakati wa utafiti, unaweza kuwasiliana na wafuatao: DKT. Winnie Mueni Saumu, chuo kikuu cha nairobi, Simu ya mkono 0723317632 ***AU*** mwenyekiti, knh/uon kamati inayoshughulikia maadili, Nambari ya simu: 726300-9, ext. 44355 or 44102 Nairobi ama uandike barua pepe kwa uonknh_erc@uonbi.ac.ke au barua kupitia Sanduku la posta, 20723-00202, Nairobi.

CONSENT CERTIFICATE IN SWAHILI

CHETI CHA IDHINI

Kabla sijakuhusisha katika utafiti wangu, naomba utie sahihi katika fomu ya idhini iliyopo hapo chini. Fomu hii ya idhini haitahusishwa na majibu yako. Sahihi hii inahakikisha kuwa umekubali kushiriki kwa utafiti huu pamoja na mwanao.

Kauli ya ridhaa: Nimesoma habari hapo juu na nimepata majibu ya maswali yoyote

SAHIHI……TAREHE……

JINA …….

MKUU WA UCHUNGUZI

SAHIHI ……TAREHE……

JINA ……

Ahadi ya mhusika

Ninakiri ya kwamba nimeelezwa na kufafanuliwa muktadha wa utafiti huu, na nimeelewa ya kwamba ni hiari yangu kuhusika. Pia, nimeelewa ya kwamba naweza kujiondoa kwenye utafiti huu wakati wowote bila kuhatarisha matibabu yangu. Aidha, nimeelewa ya kwamba maswali yote nitakayozua kuhusiana na utafiti huu yatajibiwa na mtafiti mkuu, Dkt Winnie Mueni Saumu kupitia simu ya rununu) 0723317632, au barua pepe saumubundi@gmail.com. Pia, ninaweza kuwasiliana na kamati ya Maadili ya Hospitali ya Kenyatta na Chuo Kikuu cha Nairobi kupitia nambari 726300-9, ext. 44355 or 44102 Nairobi ama uandike barua pepe kwa

uonknh_erc@uonbi.ac.ke au barua kupitia Sanduku la posta, 20723, Nairobi.

Mkuu wa uchunguzi

Dr Winnie Mueni Saumu

Chuo Kikuu cha Nairobi, P.O. Box 30197-00100

Barua pepe; saumubundi@gmail.com, Simu: 0723317632

Msimamizi mkuu wa uchunguzi:

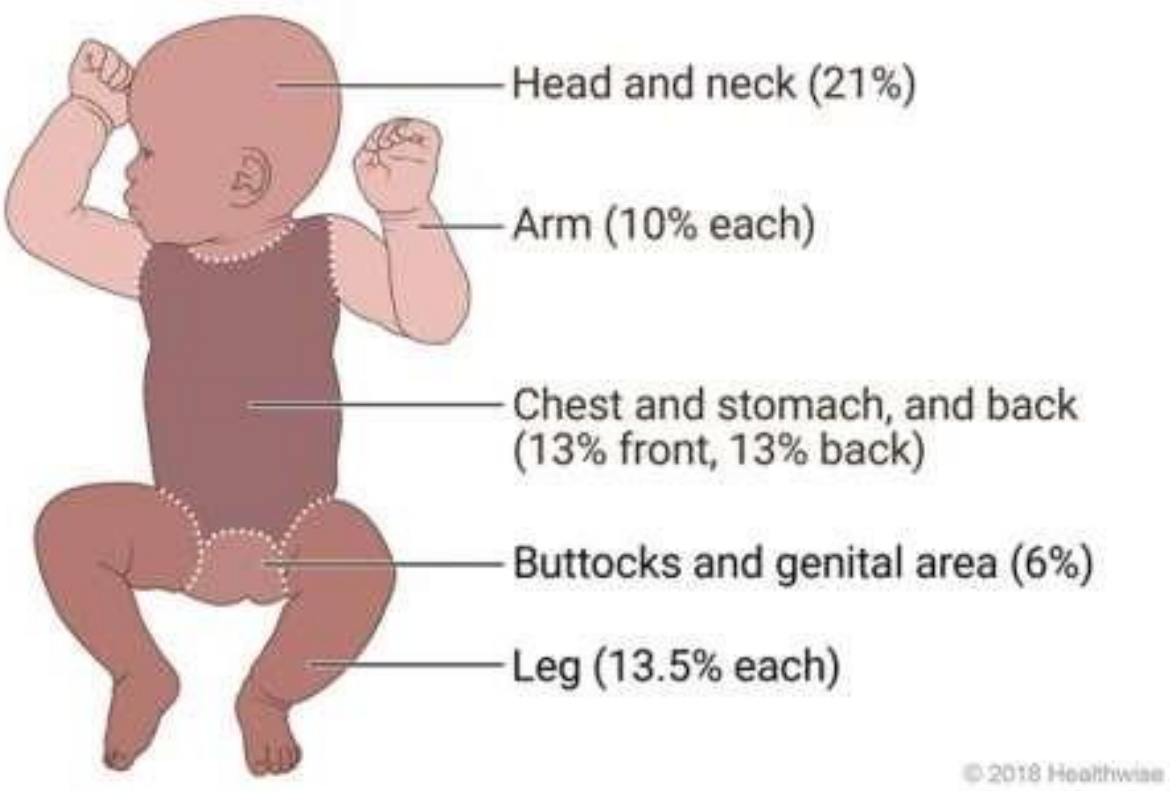
Dkt. Lucy Mungai

Chuo Kikuu cha Nairobi, P.O. Box 30197-00100

Barua pepe: dr.lmungai@gmail.com Simu: 0724654135

APPENDIX IV: TOOLS TO BE USED IN THE STUDY

LUND BROWDER INFANT CHART FOR ASSESSMENT OF BODY SURFACE AREA EXPOSED TO SUNLIGHT



BRISTOL BREASTFEEDING VALIDATION TOOL

	0 Poor	1 Moderate	2 Good	Score
POSITIONING **Baby well supported; Tucked against mother's body; Lying on side /neck not twisted; Nose to nipple; Mother confident handling baby**	No or few elements achieved **Needs to be talked through positioning**	Achieving some elements **Some positioning advice still needed**	Achieving all elements **No positioning advice needed**	
ATTACHMENT **Positive rooting; Wide open mouth; Baby achieving quick latch with a good amount of breast tissue in mouth; Baby stays attached with a good latch throughout feed**	Baby unable to latch onto breast or achieves poor latch. No/few elements achieved **Needs to be talked through attachment**	Achieving some elements **Some advice on attachment needed**	Achieving all elements **No advice on attachment needed**	
SUCKING **Able to establish effective sucking pattern on both breasts (initial rapid sucks then slower sucks with pauses). Baby ends feed.**	No effective sucking; no sucking pattern	Some effective sucking; no satisfactory sucking pattern; on and off the breast	Effective sucking pattern achieved	
SWALLOWING **Audible, regular soft swallowing- no clicking**	No swallowing heard; clicking noises	Occasional swallowing heard; some swallows noisy or clicking	Regular, audible, quiet swallowing	

PAMPHLET ON IMPORTANCE OF VITAMIN D (ENGLISH VERSION)

Why is vitamin D important for babies and children?

Babies and children need vitamin D to grow normally and develop healthy bones.

What can happen if babies and children don't get enough vitamin D?

Babies and children who don't get enough vitamin D can get a condition called "rickets." Rickets can make bones thin and weak. Some children with rickets have legs that bend to the side, called "bow-legs"

Is my baby or child at risk of getting too little vitamin D?

Babies and children can be at risk if they:

- Do not drink enough milk or eat other foods with vitamin D in them or their mothers have vitamin D deficiency
- Have dark skin
- Spend most of their time inside or live in a place with little sun
- Were born premature
- Take certain medicines
- Have a medical condition that makes it hard to get enough vitamin D, such as cystic fibrosis or celiac disease

How can I make sure my baby or child gets enough vitamin D?

To get enough vitamin D for healthy bones, most babies and children need to:

Have plenty of drinks or foods that have vitamin D added. (This is called "fortified.") The most common ones are milk, yogurt, and baby formula.

Ensure adequate sun exposure

Or, take a vitamin D supplement. Supplements are liquids, pills, or capsules that have nutrients in them.

Is there a test for vitamin D?

Yes. Your child's doctor or nurse can do a blood test to check your child's vitamin D levels. If levels are low, the child is started on supplementation

A. SWAHILI VERSION: UMUHIMU WA VITAMINI D

Kwa nini watoto wanahitaji vitamin D?

Watoto wanahitaji vitamini D ili wawe na afya nzuri na mifupa yao inawiri.

Nini inaweza kutokea watoto wakipungukiwa na madini ya vitamin D?

Watoto wakikosa vitamin D wanaweza kupatwa na ugonjwa wa matege. Ugonjwa huu usababisha mifupa kuwa laini na dhaifu. Watoto walio na matege huwa na miguu iliyojipinda au kombo.

Je, mtoto wangu ako kwa hatari ya kupungukiwa na madini ya vitamini D?

Watoto walio kwa hatari kubwa ya kukosa madini ya vitamin D ni wafuatao :-

- Watoto wenye hawapati maziwa ya kutosha au vyakula vyenye madini ya vitamin D ama mama yao anakosa madini haya.
- Watoto wenye ngozi nyeusi
- Watoto wenye hawapati mwangaza wa kutosha wa jua
- Watoto waliozaliwa mapema, kabla ya siku zao za kuzaliwa kufika
- Watoto wanaotumia madawa kama vile ya kutibu ugonjwa wa kifafa ama kifua kikuu
- Watoto walio na magonjwa ambayo inafanya madini haya kupungua kwa mwili kama vile ugonjwa wa figo, maini ama ya utumbo.

Nitahakikisha aje kuwa mwanangu ana madini ya kutosha ya Vitamini D?

Mtoto wako anaweza kupata madini ya vitamin D kwa njia zifuatazo:-

- Hakikisha amepata chakula ama vinywaji vilivyoongezewa vitamini D kama vile Maziwa, maziwa ya kopo na maziwa lala baada ya miezi sita.
- Kuweka mtoto wako kwenye mwanga wa jua kwa kadiri ya dakika thelathini kila siku

- Ama kumpea dawa ya kuongezea madini ya vitamin D

Kunao kipimo cha madini ya vitamini D?

Ndio, Daktari ama muuguzi wako anaweza kufanyia mtoto wako kipimo ili kubainisha kiwango chake cha madini ya Vitamini D. Mtoto wako akipatikana na upungufu wa madini haya, anaweza kupewa dawa ya kuongezea vitamin D kwa mwili.

APPENDIX V: SOP ON SAMPLE COLLECTION FOR THE MOTHERS AND INFANTS.

1. The research assistant will assemble all the supplies needed for the procedure in the consultation room. These include:- alcohol swabs, tape, cotton wool, needles, syringes, tourniquet, needle disposal unit and biohazard disposal bins.
2. The researcher will introduce him/herself to the study participant and explain the procedure.
3. Verification of the participants unique identification number will be done to ensure the unique number used to label the blood collection tubes of the mother and the infant corresponds with their unique identification number on the questionnaire. Date and time will be indicated on the label too.
4. The researcher will wash their hands and wear appropriate personal protection Equipment. Minimum standard will be a labcoat and a pair of disposable gloves.
5. The infant will be held in a comfortable position on the lap of the mother or placed on a comfortable examination couch.

NB: For the mothers, they will sit comfortably on a Chair.

6. The mothers will have their blood samples drawn 1st, then the infant's.
7. Tourniquet will be applied
8. The best site for the procedure will be identified by palpation of the veins.

9. The identified site will be cleaned with alcohol swabs in a circular motion starting from inside going outwards and allow alcohol to dry.
10. With the Non-dominant hand, the site of puncture will be stablilised and the researcher will use the dominant hand to puncture the skin at a 30^0 angle using a needle and a 5 cc size syringe.
11. The collected samples will be transferred to the appropriate vacutainer
12. If blood flows freely, the tourniquet may be loosened. Remove the tourniquet just before the last blood sample has been obtained. Do not leave the tourniquet on for more than 1 minute.
13. Apply a clean gauze pad over the puncture site and withdraw the needle. Caution must be observed to prevent from getting stuck with a contaminated needle.
14. Apply the needle safety device (follow manufacture instructions for specific device used) and discard the needle with the safety device in place into the sharps container.
15. Invert blood collection tubes. Do not shake.
16. Apply pressure to the site for approximately 2 to 3 minutes.
17. Tape the gauze pad or a band-aid may be applied to the site.
18. Discard blood contaminated products into the biohazard trash and discard the needles in the sharps box.
19. Verify the unique identification number on the label in the presence of the subject before taking the sample to the lab.

APPENDIX VI: TOOLS AND SOP FOR ANTHROPOMETRIC MEASUREMENTS OF INFANTS.

A. TOOLS TO BE USED

Infantometer for checking the length

Seca 376 digital weighing scale for checking the weight

Tape measure for checking the head circumference

Cotton wool swabs for cleaning the scale

Clinical paper roll

Heater to keep the room warm

B. SOP FOR WEIGHT MEASUREMENT

Before weighing the baby

Place the weighing machine on a flat surface

Clean the pan/ tray of the weighing scale with alcohol swabs

Place a clean clinical paper roll on the pan/ tray

Calibrate the scale into kilograms and to a reading of zero

Sanitize hands

Explain the procedure to the mother

During the procedure

Request the mum to fully undress the infant and place the infant slowly in the middle of the weighing scale's pan

Note the weight of the infant when it shows a stable reading.

Recalibrate the scale to zero and take a second reading.

Handover the baby to the mother to be dressed.

Take the mean of the two readings and record in the questionnaire in kilograms

After procedure

Replace the articles ready for weighing of the next infant

Discard the used items in the appropriately colour coded waste bins (black coded for non- infectious waste).

C. SOP FOR LENGTH MEASUREMENT

Before the procedure

- Place the infantometer on a flat surface
- Clean it with alcohol swabs
- Sanitise hands

- Explain the procedure to the mother

During the procedure

Two assistants are required.

- Place the baby supine on the infantometer
- Head of the baby should touch the fixed headboard (one assistant stabilizes the head against the footboard)
- Feet of the baby should touch the foot board. (2^{nd} assistant adjusts the movable foot board)
- During the procedure, ensure the infant is relaxed with the legs fully extended at the knee level and the head positioned in frankfurt plane (meaning the imaginary line connecting the outer canthus of the eye and the external auditory meatus is perpendicular to the long axis of the trunk.
- The length measurement to be repeated thrice and the mean of the three readings recorded on the questionnaire to the nearest 0.5 cm.

After the procedure

Clean the infantometer and sanitize hands ready for the next infant.

Weight for Height Z-scores

After accurate weight and height are recorded, the Z-score shall be calculated and recorded on the questionnaire.

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P O BOX 20723 Code 00202
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Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/188

12th May, 2023

Dr. Winnie Mueni Saumu
Reg.No.H121/41439/2021
Fellow in Paediatric & Adolescent Endocrinology
Dept. of Paediatrics & Child Health
Faculty of Health Sciences
University of Nairobi

Dear Dr. Saumu,

ETHICAL APPROVAL-RESEARCH PROPOSAL: VITAMIN D STATUS AND MAGNESIUM STATUS AMONG INFANTS AGED 6 WEEKS TO 12 MONTHS ATTENDING CHUKA COUNTY REFERRAL HOSPITAL WELL BABY CLINIC (P930/12/2022)

This is to inform you that KNH-UoN ERC has reviewed and approved your above research proposal. Your application approval number is **P930/12/2022.** The approval period is 12th May 2023 – 11th May 2024.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by KNH-UoN ERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to KNH-UoN ERC 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected 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.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. 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.
vii. Submission of an executive summary report within 90 days upon completion of the study to KNH-UoN ERC.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

DR. BEATRICE K.M. AMUGUNE
SECRETARY, KNH- UoN ERC

c.c. The Dean, Faculty of Health Sciences, UoN
The Senior Director, CS, KNH
The Chairperson, KNH- UoN ERC
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