PREVALENCE OF DISTURBANCE IN CALCIUM METABOLISM AMONG PAEDIATRIC PATIENTS ON LONGTERM ANTICONVULSANTS AT MOI TEACHING AND REFERRAL HOSPITAL ELDORET, KENYA

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE, CHILD HEALTH AND PAEDIATRICS, MOI UNIVERSITY

DECLARATION

This thesis is my original work and has not been presented before for another degree in any other University/Institution.

……. ……..

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DEDICATION

I would like to dedicate this work to my parents Mr and Mrs J.M Kinara, for always supporting my dreams and ambitions and my siblings for encouraging me each step of the way.

ACKNOWLEDGEMENT

I wish to sincerely thank my supervisors Dr Paul Kiptoon, Dr Eren Oyungu and Dr Justus Simba for their input and guidance during the process of proposal development, execution of the study, data analysis, presentation and finally writing of the book for submission. I also wish to thank the staff at the Pediatrics Neurology clinic, participants and their guardians without whom this study would not be possible. Last I also appreciate my lecturers and colleagues in the Child Health and Pediatrics Department of Moi University for the corrections and moral support.

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ABSTRACT

Background: Epilepsy is the most prevalent neurological disorder in children. Treatment of epilepsy involves the use of anticonvulsant drugs over a long duration with the aim of remission or when not possible, a reduction in the frequency of seizures. Anticonvulsant medications are associated with undesirable side effects that may be life-threatening or interfere with function. Disturbance in calcium metabolism leading to hypocalcaemia is one of the side effects of anticonvulsants, especially cytochrome p450 inducers which may eventually lead to break-through seizures or predispose to fractures. There is no local data on the prevalence of hypocalcaemia or consensus on the need for testing of calcium levels among children on anticonvulsant drugs. This study aims to describe the magnitude of hypocalcaemia among children on anticonvulsants drugs in our local population.

Objective: To determine the prevalence and factors associated with derangement in calcium metabolism among children on chronic anticonvulsant drugs attending the neurology clinic at Moi Teaching and Referral Hospital.

Methods: This was a cross-sectional study conducted over nine months at MTRH paediatric neurology clinic. Consecutive sampling was done and children aged six months to 14 years who had been on anticonvulsants for at least six months were recruited. Data on the socio-demographic and clinical characteristics of the participants was collected using an interviewer guided questionnaire. Venous blood was analysed for calcium, phosphate, and alkaline phosphatase levels, which were considered markers for calcium metabolism. The prevalence of derangements in calcium metabolism was presented using frequency distribution. Chi-square and Fischer's test were used to test for associations, and all analysis was at 95% CI.

Results: A total of 98 patients were recruited with a male: female ratio of 1.2:1 and a median age of 8yrs (IQR 4, 11). The most common type of epilepsy was generalized tonic-clonic seizures at 38.8 %. Length of anticonvulsive therapy ranged from 6-96 months, mean 17.6 months (SD 16.9) with 56% of the patients being on monotherapy. Hypocalcaemia, hypophosphataemia, and raised alkaline phosphatase were found in 13.3%, 12%, and 35% respectively. However, none of the patients had combined derangements in all three parameters. There was no significant association between demographic and clinical factors with hypocalcaemia; gender (P=0.306), duration of anticonvulsants use (P=0.64) and use of enzyme-inducing anticonvulsant drugs (P=0.533). There was no significant association of hypophosphatemia with; gender (P=0.65), duration of AED use (P=0.507) or use of enzyme-inducing anticonvulsant drugs (P=0.055).

Conclusion: The study found a tenth of children with hypocalcemia and hypophosphatemia while 1/3 had raised alkaline phospatase. Calcium metabolism disturbance was not significantly associated with the clinical factors of the epileptic children enrolled in the study.

Recommendations: Future studies that are larger with more drug representation to establish their influence on calcium and any associated factors

ABBREVIATIONS

25(OH) D	25-hydroxyvitamin D
AED	Antiepileptic drugs
ALP	Alkaline phosphatase
IREC	Institutional Research and Ethics Committee
MTRH	Moi Teaching and Referral Hospital
PTH	Parathyroid hormone

OPERATIONAL DEFINITIONS

Disturbance in parameters of calcium metabolism defined as; calcium <2.3mmol/l with phosphate <0.85mmol/l or increased alkaline phosphatase for the age group.

Chronic anticonvulsant treatment -use of a specific anticonvulsant drug for more than 6 months.

Cytochrome p450 inducing antiepileptic drugs- phenytoin, phenobarbital, carbamazepine.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Epilepsy is considered as one of the commonest neurological disorder in the world and WHO estimates that about 70 million people suffer from it. Children below 15 years account for 25% of those affected and 40% of the new epilepsy cases annually (WHO, 2004). In Kenya, a study by (Mung'ala et al.2008) estimated the prevalence of active epilepsy in children aged 6-12 years at 11/1000 while another study found the incidence of active epilepsy highest in this age group at 96.1/100,000 persons per year (Anthony K. Ngugi, et al., 2013) .

A number of medications are used in the treatment of epilepsy. The older antiepileptic drugs include phenobarbital, phenytoin, carbamazepine, primidone, sodium valproate and clonazepam. Newer antiepileptic drugs include lamotrigine, topiramate, oxcarbazepine, clobazam and levetiracitam. In the developing countries epileptic children are mainly on the older antiepileptic drugs which are cheaper and more readily available than the newer drugs. The Kenya National Guideline for epilepsy management 2016 recommends phenytoin, carbamazepine, sodium valproate and phenobarbital for use in primary level facilities which also cater for a majority of the epileptic patients.

Anti-epileptic drugs have long been linked with both hematological disorders and bone metabolic disorders. Most of the early studies associated deranged calcium metabolism with patients taking CYP450 inducing drugs therefore majority of the published studies and evidence is from patients on these drugs. It is postulated that CYP450 enzyme induction leads to increased catabolism of vitamin D, resulting in decreased absorption of calcium, secondary hyperparathyroidism, and decreased bone mineral density.

Children suffering from epilepsy also frequently present with other co-morbid conditions including anaemia, malnutrition and depressed cellular immunity while a good number may be immobile or institutionalised. In the above setting these children are therefore at a higher risk of suffering from drug related adverse effects which include abnormal calcium metabolism.

Hypocalcemia is often insidious and asymptomatic but may be severe as well. It is also often accompanied by low phosphate levels and high alkaline phosphatase as these two are usually affected when the body is trying to maintain eucalcemia.

Clinicians therefore need to be aware of the adverse effect of AEDs on calcium metabolism in order to detect it early and prevent further complications which may include osteomalacia and increased risk of fractures (Cassandra V et al., 2010).

This study aimed to look at the prevalence of hypocalcemia among the epileptic patients on anticonvulsants in the Kenyan setting. This information will be important in formulating protocols on proper follow-up in terms of screening for hypocalcemia and calcium supplementation among these patients.

1.2 Problem Statement

Epilepsy treatment often requires chronic or lifelong use of anticonvulsants. With this in mind it is important for clinicians to consider the various adverse effects associated with long term use of these drugs during initiation of treatment. Various studies have been done to describe the effect of these drugs on bone metabolism and hypocalcemia. One of the earliest studies was by K De Luca et al. (1972) in a case control study that demonstrated a significant disturbance in calcium metabolism in the children on AEDs compared to the controls. Although findings are varied there is current evidence that still supports the theory that long term AEDs causes a significant drop in 25(OH) D,

hypocalcemia with a resultant hypophosphatemia and decreased bone mineral density (Menon B et al., 2010).They have also been linked with an increased risk of fractures with those who have used them for more than 12 years being at greatest risk. (Souverein PC et al., 2006).

A survey on practice pattern of neurologists regarding bone effects of AEDs found that only 41% of pediatric and 28% of adult neurologists reported screening their AED-treated patients for bone and mineral disease. Of paediatric neurologists who detected bone disease through diagnostic testing, 40% prescribed calcium or vitamin D, and about 54% referred patients to specialists. Only a small number of those neurologists interviewed prescribe prophylactic calcium or vitamin D for patients taking AEDs. The conclusion was most neurologists do not consider AED induced bone disease of clinical importance while there are some who may not be aware of this association. (Cassandra V. et al., 2010).

There are no set guidelines on whether to supplement calcium and vitamin D in a patient with epilepsy from day of initiation of AED therapy or whether a baseline data of markers of bone formation, resorption, and calcium and vitamin D levels are mandatory. It is therefore important for more studies to be done in our setting to determine the effect of anticonvulsants on bone and calcium metabolism in order to increase awareness on this adverse effect and promote osteoprotective practices in health care providers.

1.3 Justification

Adverse effects of AEDs on bone metabolism were first discovered decades ago and since then studies have been done to establish the mechanism and magnitude of these electrolyte and hematological side effects. Earlier studies were focused on hypocalcemia in institutionalised or retarded children (Tolman KG et al., 1975). Although hypocalcemia and associated vitamin D deficiency have been mainly associated with institutionalised patients recent evidence has shown that even ambulatory patients on these drugs may experience these derangements (Bouriask P et al., 2013.). Calcium is also an important ion for neurotransmission and hypocalcemia in epileptic patients on anticonvulsants has been linked to poor seizure control despite compliance on medication (Ali et al, 2004). Calcium and vitamin D supplementation has therefore been recommended among patients on chronic anticonvulsants (Collins N et al., 1991).

Calcium, phosphorous and alkaline phosphatase are important indicators for bone health, the laboratory tests are also available in most of the county and sub county hospitals throughout the country. Despite evidence from other studies showing derangements in calcium, phosphorous and ALP among patients on chronic anticonvulsants there are no local studies that have been done in this field.

This study aims to provide an understanding of the prevalence of hypocalcemia among these patients on chronic anticonvulsants. This information will aid in monitoring patients at risk and planning of appropriate prophylactic and therapeutic measures. Since these laboratory tests are relatively available throughout the country, clinicians can therefore improve follow up and treatment of the epileptic patients in their care.

1.4 Research Question

What is the prevalence of disturbance in calcium metabolism among children with epilepsy on chronic anticonvulsants at Moi Teaching and Referral Hospital?

1.5 Broad Objective

To determine the prevalence of disturbances in calcium metabolism among paediatric epileptic patients on chronic anticonvulsants attending the neurology clinic at Moi Teaching and Referral Hospital.

1.6 Specific Objectives

1. To determine the prevalence of derangements in calcium, phosphate and alkaline phosphatase levels among epileptic patients on chronic anticonvulsant drugs attending the paediatric neurology clinic at MTRH.
2. To describe the clinical factors associated with derangements in calcium, phosphate and alkaline phosphatase levels among epileptic children on chronic anticonvulsant drugs attending the paediatric neurology clinic at MTRH.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Regulation of calcium, phosphate and Alkaline Phosphatase

Calcium is the third most abundant ion in the body and it plays a key role in normal cell function, membrane stability, neural transmission, intracellular signaling, bone structure and blood coagulation. Serum calcium levels are tightly controlled within a narrow range, usually 8.5–10.5 mg/dL (2.1–2.6 mmol/L). Serum calcium levels account for only 0.1–0.2% of extracellular calcium, which in turn is about 1% of total body calcium. The remainder of total body calcium is usually stored in bone. Ionized calcium is physiologically active and is about 40% of total serum calcium level, while the non-ionized calcium is bound to albumin or anions such as phosphorus, citrate and bicarbonate. Hypocalcemia is therefore defined as total serum calcium lower than 8.5mg/dl or ionized serum calcium lower than 4.7mg/dl. A decrease in extracellular (ECF) calcium causes an increase in parathyroid hormone (PTH) secretion through activation of the calcium sensor receptor on parathyroid cells. PTH will in turn, enhance renal tubular reabsorption of calcium, stimulate renal 1,25-dihydroxycholecalciferol [1,25(OH)2D3] production and increase calcium resorption from bone, (Boden SD Kaplan FS, 1990).

Vitamin D also plays an important role in calcium control by stimulating intestinal absorption of calcium, regulating PTH release by the chief cells, and also mediating PTH-stimulated bone reabsorption. Collectively, these homeostatic mechanisms ensure that calcium levels are maintained within the normal range. Hypocalcemia will occur when there is a net efflux of calcium from the extracellular fluid exceeding what can be replaced by the intestine or bone as a result of any disruption of the aforementioned regulatory mechanisms (Gregory R Mundy et al, 1999).

Inorganic phosphorus is important in numerous physiologic functions including skeletal development, mineral metabolism, cell signaling, cell membrane phospholipid content and function, energy transfer through mitochondrial metabolism and platelet aggregation. Because of its importance, normal homeostasis maintains serum concentrations between 2.5 to 4.5 mg/dl (0.81 to 1.45mmol/L). The terms phosphorus and phosphate are often used interchangeably, but the term phosphate means the inorganic freely available form (HPO_4^{-2} to $H2PO_4^{-1}$). Phosphorus absorption is dependent on both passive transport related to the concentration in the intestinal lumen (i.e. increased after a meal) and active transport stimulated by calcitriol. Medications or foods that bind phosphorus (antacids, phosphate binders, calcium) can decrease the net amount of phosphorus absorbed by decreasing the free phosphate for absorption. When there is a decrease in serum phosphate level, there is stimulation of the 1-alpha hydroxylase enzyme in the kidneys which increases conversion of calcidiol to calcitriol which in turn increases intestinal phosphorus absorption. There is also a reduction in urinary phosphorus excretion.

Alkaline Phosphatases are a group of enzymes found mainly the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2). There are also small amounts produced by intestinal cells (isoenzyme ALP-3), the placenta, and the kidney (in the proximal convoluted tubules). When serum ALP is measured, it is the total amount of alkaline phosphatases released from these tissues into the blood. As the name suggests, this enzyme works best at an alkaline pH (a pH of 10), and therefore the enzyme itself is inactive in the blood. Alkaline phosphatases act by splitting off phosphorus (an acidic mineral) to create an alkaline pH. ALP is also important in mineralization of bones. Serum ALP measurements are of particular interest in the investigation of hepatobiliary and bone diseases.

2.2 Mechanisms of Anticonvulsant drugs associated disturbance in calcium metabolism

Since the discovery of an association between AEDs and bone disease there have been several theories that have been proposed to explain this phenomenon. The principle mechanism noted is hepatic induction of cytochrome p450 enzyme system leading to catabolism of vitamin D. Phenobarbital, phenytoin, and carbamazepine are among a class of drugs known as xenobiotics. Xenobiotics activate a nuclear receptor known as either the steroid and xenobiotic receptor (SXR) or pregnane X receptor (PXR). On the molecular level they activate the orphan nuclear receptor, pregnane X receptor (PXR), which shares 60% homology in their DNA-binding domains to the vitamin D receptor (VDR), and is expressed in the intestine, kidney, and liver. The PXR has been shown to mediate induction of CYP2 and CYP3, the cytochrome P450 enzymes involved in drug metabolism. Emerging evidence shows that these PXR activators can increase the expression of the CYP24, a VDR target gene, in cultured cells and in vivo in mice. CYP 24 is an enzyme that directs the side chain oxidation and cleavage of 25(OH) 2 D3 and 1ß, 25 (OH) 2D3 to carboxylic acid end products (calcitroic acid), resulting in lower cellular concentration of active vitamin D (Valsamis et al., 2006). This increased conversion of vitamin D to inactive metabolites will decrease bioavailable vitamin D which in turn decreases biologically active vitamin D leading to reduced absorption of calcium in the gut resulting in hypocalcemia and increased PTH. Increase in PTH will subsequently lead to mobilization of bone calcium stores and subsequent bone turnover resulting in an elevation in serum ALP. (Pack et al., 2008)

Antiepileptic drugs may also interfere with absorption of calcium in the gut leading to hypocalcemia and feedback hypersecretion of PTH. Koch et al. (1972) was able to

demonstrate this in rats where those treated with phenytoin had markedly decreased calcium absorption compared to the phenobarbital treated group.

Hahn et.al. (1972) studied fetal rats treated with phenytoin and phenobarbital, they showed impaired response to PTH. This inhibited cellular response to PTH could lead to the hypocalcemia seen in patients on AEDs.

Hyperparathyroidism could be another possible mechanism. Patients with normal vitamin D levels and those who had low vitamin D had hyperpaparathyroidism. These high levels of PTH primarily affect bone resorption.

Sodium valproate does not induce hepatic p450 enzymes and interfere with absorption of calcium or phosphate like phenobarbital and phenytoin yet it has been shown to still cause hypocalcemia and affect bone mineral density as demonstrated by Borusiak et al., 2012. Sodium valproate is postulated to cause hypocalcemia by direct or indirect (through hyperleptinemia) effects on the calcium sensing receptor causing hypocalcemia. It may also cause renal tubular dysfunction with increased urinary loss of calcium and phosphate known as reversible Fanconi (Ecevit et al., 2004).

Final theorized mechanism is calcitonin deficiency. Calcitonin is a hormone that is produced by the thyroid gland. It inhibits osteoclast mediated bone resorption. Deficiency may therefore accelerate bone turnover (Kruse K et al., 1987).

2.3 Prevalence and clinical factors Associated with Calcium, phosphate and Alkaline Phosphatase Disturbance

The effects of antiepileptic drugs on bone health have been described since 1960s. In earlier studies they reported severe bone disease described in pathological biopsies. However, these studies were performed predominantly in institutionalized patients in whom confounding variables such as inadequate sunlight exposure, poor diet, and limited exercise possibly influenced the findings. Recent studies in ambulatory persons describe radiographic and biochemical abnormalities consistent with decreased bone mineral density (BMD) and disorders of bone mineral metabolism. There are varying findings on the impact of anticonvulsants on bone density, growth and biochemical derangements even in more recent studies. Several studies on this topic have been conducted over the years but due to the differences in populations, climate, calcium intake, study designs and sizes it is difficult to make a conclusion on the magnitude of this effect and the various contributing factors. Hypocalcemia in those using AEDs varies between 3-45%. Decreased serum phosphate has also been described in a number of studies.

In India, Seth et al (2017) reported hypocalcaemia in 42.5% children with cerebral palsy and epilepsy. In Britain (Hunter et al., 1971) looked at 105 epileptic children aged 10 to 16 years and found hypocalcaemia in 30% and raised alkaline phosphatase activity in 24%. In another study (Richens & Rowe, 1970) looked at 160 epileptic patients in a residential center and reported that 22.5% of all the participants had hypocalcaemia. Borusiak et al. (2012) performed a multicenter cross sectional study among children who had been on valproate, lamotrigine, sulthiame, levetiracetam or topiramate for at least 6 months. Among 128 patients, 24.4% had hypocalcemia 13.3% low 25 (OH)

vitamins and 25.4 % hypophosphatemia. All the patients were however clinically asymptomatic. They also found that mean calcium concentrations in this group were significantly lower than the healthy controls.

In another Turkish study by Ecevit et al they found a hypophosphatemia prevalence of 50% compared to 17.6% of hypocalcaemia among children who had been on valproate monotherapy.

Other studies have reported lower prevalence of hypocalcemia and hypophosphatemia. In Iran Nakhaeymoghadam et al reported a 7.5% prevalence of hypocalcemia while no patient had hypophosphatemia among 40 children in the outpatient department. In Malaysia Yi et al reported that only 3% of the children on both enzyme and non-enzyme inducing drugs had hypocalcemia. Ojinnaka et al however reported no abnormality in calcium, phosphate and alkaline phosphatase among children on phenobarbitone monotherapy (Ojinnaka N & Ileoje S 1997). This finding was also similar with Babayigit et al in Turkey who also found no patient with hypocalcemia or hypophosphatemia but the levels of Alkaline phosphatase were higher among those on anticonvulsants compared to the controls (Babayigit et al., 2006)

Several markers of bone formation have also been assessed in those taking AEDs including osteocalcin, ALP, and C termina extension peptide of type 1 procollagen. Of all these ALP is commonly used and elevations are often seen in both children and adults on AEDs (Bogliun et al.,1986.) Although it may be argued that total ALP is from bone liver and other sources, Okesina et al. (1991) measured the isoenzymes among these patients and reported that the increase in total ALP was mainly due to bone fraction. In a study by Crosley et al at a paediatric outpatient unit over a 12 month period, he found increased ALP in 42% of the 74 children while derangements in

calcium and phosphate were 5% and 12% respectively (Crosley et al., 1975). In Malaysia there were similar findings where Yi et al reported hypocalcemia at 3% while increased ALP was at 37.9% (Yi C et al., 2017). There are several studies that report no changes in calcium and phosphate but report increased ALP in those taking anticonvulsants compared to the controls (Kafali et al., 1999; Babayigit et al., 2006; Winnacker J et al., 1975)

There have been no reports of an association between sex and incidence of hypocalcemia although age has been found significant, (Oner et al 2004) found that the osteopenic patients in their study were younger than the non osteopenic ones.

Richens & Rowe (1970) in a survey of epileptic patients in a residential home found that there was a strong association between hypocalcemia and high dosage of drugs, multiple drug therapy and pheneturide had the highest incidence of hypocalcemia.

Pack et al. (2005) demonstrated that patients on monotherapy with carbamazepine, phenytoin and sodium valproate had significantly lowered serum calcium compared to lamotrigine. While ALP was significantly lower in those taking phenytoin than the other three (carbamazepine, valproate and lamotrigine).

In a comparison between carbamazepine phenytoin, phenobarbital and sodium valproate the degree of severity of hypocalcemia depended on the type of drug with phenobarbital being the worst compared to carbamazepine and phenytoin while no patient on valproate had hypocalcemia. (Gough et al. 1986). Longer duration of AEDs and higher doses have also been attributed with a higher incidence of osteopenia. (Oner et al. 2004)

Chronic treatment regime on anticonvulsants and polytherapy have been reported to compromise bone mass (Petty et al., 2005). Polytherapy (p-value <0.001) and enzyme-inducing anticonvulsant drugs (p-value <0.001) was shown to be significantly associated with detrimental effects on calcium serum levels (Tombini et al., 2018). Another study conducted in Lebanon was able to demonstrate that polypharmacy and duration of drug use were significant determinants of bone mineral density and calcium concentration. The study was conducted among 42 adults and 29 children and this effect was however only demonstrated among the adult population. (Farhat G et al., 2002).

Several other studies have found no significant difference between polytherapy and duration of treatment with occurrence of these biochemical abnormalities (Bouriask P et al., 2012; Sherifa A et al., 2004; Ecevit et al., 2004).

There have been case reports of increased incidence of seizures with occurrence of hypocalcemia in patients on treatment of epilepsy, seizure control was then gained after vitamin D and calcium supplementation (Ali et al., 2008). Sherifa et al demonstrated higher calcium levels in those treated with carbamazepine and valproate compared to their untreated counterparts. From this finding it is postulated that the low level of calcium is responsible for the initiation and maintenance of convulsions (Sherifa et al., 2004).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

Cross sectional descriptive design. This design was appropriate as the study participants were only seen once and all the information collected at this encounter.

3.2 Study Site

The study was conducted at the Moi Teaching and Referral Hospital paediatric neurology clinic. The Hospital is within Eldoret town, Uasin Gishu County, which is 350 Kilometers North West of Nairobi. MTRH is a level 6 health facility serving as a teaching hospital for Moi University College of Health Sciences. Other institutions that also utilize this facility include Kenya Medical Training College (KMTC), Eldoret and University of Eastern Africa Baraton School of Nursing. MTRH is also a training center for medical, clinical and nursing officer interns. It serves as the main referral hospital for the Western part of Kenya and North Rift and has a catchment population of approximately 13 million people. The facility offers various specialized in-patient and outpatient services and apart from Paediatrics other departments include Internal Medicine, Surgery Obstetrics and Gynecology, Psychiatry among others. The paediatric neurology clinic is located in the MTRH main building. It is run by a consultant paediatric neurologist and a paediatric resident from 9:00am to 12:00pm every Thursday. The doctors attend to about 10-20 patients every clinic day of ages ranging between 2 months to 14 years.

3. 3 Study population

The study population comprised of children with convulsive disorders on chronic anticonvulsant treatment attending the outpatient neurology clinic at Moi Teaching and Referral Hospital.

3.3.1 Inclusion Criteria

- Children aged between 6 months and 14 years.
- Children who have been on any anti-epileptic drugs for more than 6 months and show good compliance by 24 hour recall or self-reported compliance.

3.3.2 Exclusion Criteria

- Children on treatment for rickets or children on calcium replacement for any other reason.
- Children on follow-up for renal or hepatic impairments

3.4 Sample size

The sample size was derived using the Fisher *et al*, (1998) formulae

$$n = \frac{Z^2 \times p \times d}{\alpha^2}$$

$$n = \frac{1.96^2 \times 0.24 \times 0.76}{0.05^2}$$

n= 283

Where;

n = sample size;

Z = the value corresponding to 95% confidence =1.96

α = Significance level at 5% = 0.05

p = prevalence of hypocalcemia among paediatric epileptic patients on chronic anticonvulsants (24.4%) (Bouriask P et al., 2013.)

d = margin of error

Correction for a finite population

There are a total of about 150 children on follow up at the neurology clinic and only 10-20 are seen on a particular clinic day. Therefore due to the finite number of patients available for recruitment the following formula was used to calculate the final sample size.

n=n/ (1+ (n/N))

283/1+ (283/150)

therefore n=98

3.5 Sampling

Consecutive sampling was done and all eligible children whose parents consented to participate in the study were recruited from the outpatient paediatric neurology clinic. This was done within the nine months of implementation of the study.

3.5.1 Study Period

The study was carried out for a nine-month duration, beginning April, 2017 to December, 2017. There were a varying number of patients every clinic day from seven to twenty four.

3.5.2 Research Instruments

The data was collected into an interviewer administered data collection tool (Appendix 4). It was structured into the following sections; socio-demographic data, clinical information and laboratory results. These sections allowed for collection of all the data required to address the objectives of this study.

3.6 Data Collection and Analysis

3.6.1 Data collection methods /study procedure

Data was collected by the principal investigator and the research assistant into the data collection tool (Appendix 4). The research assistant was a qualified Clinical Officer who was also trained on the study procedures according to the proposal's objectives. The training included proper sample collection and storage. The study participants were recruited from the triage area at the outpatient neurology clinic. The research assistant and principal investigator used the patient files to identify the neurology patients at triage, those who fit the inclusion criteria were then recruited. Informed consent was sought before recruitment into the study. The parents or guardians were taken through the study procedure and the information that was to be collected they were then required to sign the consent form before recruitment into the study. The participants were then interviewed using a structured questionnaire administered by the principle investigator or the research assistant. The interviewer administered questionnaire was used to collect information on demographic (age and sex), type of seizure, pattern of medications like type and duration of AEDs, control of seizures and a physical assessment was conducted in all subjects. Any additional information was obtained from the participants file which included type of convulsive disorder described by the clinician or on EEG where available. Majority of the children had EEG results in the file.

Assent was obtained from the children over 7 years old using the assent form (Appendix 5), they were taken through the sample collection procedure in simplified language, no coercion was used to obtain the assent and only those who assented had the sample taken.

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A venous blood sample 2mls was then drawn by the principle investigator or research assistant, it was collected in the clot activator tube BD vacutainer and transported to Precise Genomics laboratory within 1 hour to test for calcium, phosphorous and alkaline phosphatase. The sample was analysed within one hour of collection (Appendix 1). Aseptic technique was maintained throughout the collection and analysis of the sample. The laboratory results were then recorded in the study data sheet and also availed to the primary care doctor for appropriate action. Those children with hypocalcemia had their samples tested for albumin levels and the corrected calcium calculated appropriately (Appendix 2).

3.7 Quality control

All blood samples were collected by the principle investigator or the research assistant. All the samples were analysed at the Precise Genomics laboratory using the Architect C4000 machine from Abbot. Quality control was done daily using commercial controls while calibration of the machine was done weekly in accordance to the laboratory protocols. In case of failure or breakdown, there was a backup machine available.

3.8 Data Storage

Data was checked for completeness and accuracy by the principal investigator on a daily basis. Data was entered onto a prepared Microsoft Excel® sheet. Confidentiality was maintained by excluding any identifiers from the keyed dataset. The database was password protected to prevent un-authorized access. Data was backed up in a remote hard disk and flash drive to safeguard against any data loss and placed in a lockable drawer. Data cleaning was then carried out.

3. 9 Data management and analysis

The data collected was entered into an MS-excel spreadsheet then exported and analyzed using STATA version 14. Differences were considered significant at 5% α-level. Descriptive analyses was used to describe the sample such as socio-demographic characteristics such as age, gender, type of seizure and types of medication which were analyzed as frequencies. Descriptive statistics were also used to determine the prevalence of hypocalcemia and other biochemical derangements hypocalcemia which were then presented as percentages. Secondly, chi-square tests were used to identify any significant association between the deranged laboratory values and demographic factors together with various clinical factors

3.10 Ethical Considerations

Approval to carry out the study was sought and granted by the Institutional Research and Ethics Committee (IREC) Moi Teaching and Referral Hospital and the Director of MTRH.

Parents or guardians to the study participants were informed about the study, what the blood would be tested for and the benefits of the study before recruitment. No incentives were used to convince the guardians for consent to participate in the study. The participants did not pay for the laboratory tests, this cost was met by the principle investigator. The data collection tool was de-identified and did not contain the names of the participants. Confidentiality was maintained throughout the study. All patients' results were attached in their files and immediately communicated to the clinician for any medical attention to be given as necessary. The raw data collected was stored in a locked cabinet throughout the study period while the data in the computer was in a password protected file. The results shall be availed for reference at the College of

Science Resource Centre. The findings in this study will also be communicated to the clinicians in the paediatrics department and MTRH to aid in improvement of follow up of these epileptic children on chronic anticonvulsants. The results of this study shall also be availed for publication in a reputable, peer reviewed journal for access and use by the scientific and general population in the improvement of the management of patients on long term anticonvulsant use.

CHAPTER FOUR

4.0 RESULTS

4.1 Overview of the Study Population

A total of 98 children seen at the pediatric neurology clinic of Moi Teaching and Referral Hospital were enrolled into the study. Of these, 56.1% (n=55) were male; with a male to female ratio of 1:0.8. Median age was 8 years (IQR 4, 11)

4.2 Prevalence of Disturbance in Calcium Metabolism

Majority of the children, 81 had normal (corrected) serum calcium levels. Only 13 (13.3%) had hypocalcemia. The proportion of children with hypophosphatemia was 12(12.2%). The only derangement reported on serum alkaline phosphatase was high serum levels among 35(35.7%) of all the children sampled in the study (Table 4.1).

Table 4.1 Prevalence of hypocalcemia, hypophosphatemia and increased ALP

Parameter	**Number (n = 98)**
Calcium	
Low	13(13.3%)
Normal	81(80.6%)
High	4(6.1%)
Phosphate	
Low	12(12.2%)
Normal	86(87.8%)
Alkaline phosphatase	
High	35(35.71%)
Normal	63(64.29%)
***Low calcium < 2.2, Low Phosphate <0.85, Increased ALP as per age group**	

4.3 Clinical Factors Associated with Calcium Metabolism Disturbance

4.3.1 Clinical characteristics of the study population

Majority of the children (38.8%) were on treatment for generalized tonic clonic seizures. The children on monotherapy were 56%, half of whom were on treatment using carbamazepine. Length of convulsive therapy ranged from 6 months to 96 months (mean of 17.6 months, SD 16.9). Most of the children had good seizure control (62.24%) which was defined as less or equal to one seizure in 6 months. Only two children (2.04%) were on other medication one on prednisone and the other on Ritalin. None of the children had a history of fractures.

Table 4.2: Clinical characteristics of the population

Parameter	Number (n = 98)
Sex	
Female	43 (43.90%)
Male	55 (56.10%
Number of AED	
Monotherapy	55(56.12%)
Polytherapy	43(43.85%)
Type of AED in monotherapy*	**(n=55)***
Carbamazepine	28(50.9%)
Phenytoin	1(1.84%)
Phenobarbital	13(23.63%)
Sodium valproate	13(23.63%)
Type of convulsive disorder	
Simple partial	8(8.2%)
Complex partial	28(28.6%)
Secondary generalized	3(3.1%)
Absence	3(3.1%)
Myoclonic	1(1%)
Tonic	9(9.2%)
Atonic	4(4.2%)
Tonic clonic	38(38.8%)
Other	4(4.1%)
Number of convulsive episodes	
Everyday	12(12.8%)
At least once a week	8(8.5%)
At least once a month	17(18.1%)
Once in the last six months	37(39.4%)
None	24(21.3%)
Control	
Good	61(62.24%)
Poor	37(37.76%)
Other medication	
Yes	2(2%)
No	96(98%)
History of fracture	
Yes	0
No	98(100%)

4.3.2 Clinical Factors Associated with Calcium Metabolism Disturbance

When the clinical factors were compared to hypocalcemia, hypophosphatemia and elevated alkaline phosphatase, there was no statistically significant association found (Tables 4.3, 4.4 and 4.5).

Table 4.3: Univariate test of association between clinical factors and hypocalcemia

Parameter	**Participants (n=98)**	**Low (n=13)**	**Normal (n=85)**	**p Value (X^2)**
Sex				
Female	43	4 (9.30%)	39(81.70%)	0.306
Male	55	9 (16.36%)	46 (83.64%)	
Duration				
<24 months	73	9 (12.33%)	64 (87.67%)	0.64
>24 months	25	4 (16.0%)	21 (84.0%)	
Type of convulsive disorder[1]				
Focal	36	2(5.56%)	34 (94.44%)	0.934
Generalized	62	9(13.19%)	53(86.81%)	
Control[2]				
Poor	37	4 (10.81%)	33 (89.19%)	0.577
Good	61	9(14.75%)	52 (85.25%)	
Therapy (Number)				
Monotherapy	55	7 (12.73%)	48 (87.27%)	0.859
Polytherapy	43	6 (13.95%)	37 (86.05%)	
Enzyme inducing[3]				
Non-enzyme	13	1 (7.69%)	12 (92.31%)	0.533
Enzyme	42	6 (14.29%)	36 (86.71%)	

1. Classification according to ILAE 2017
2. Good control defined as less or equal to one seizure in 6 months
3. Non enzyme inducing drug was sodium valproate

Table 4.4: Univariate test of association between clinical factors and hypophosphatemia

Parameter	Participants (n=98)	Low (n=12)	Normal (n=86)	p Value (X^2)
Sex				
Female	43	6 (13.95%)	37(86.05%)	0.648
Male	55	6 (10.91%)	49 (89.09%)	
Duration				
<24 months	73	8 (10.96%)	65 (89.04%)	0.507
>24 months	25	4 (16.0%)	21 (84.0%)	
Type of convulsive disorder[1]				
Focal	36	2(5.56%)	34 (94.44%)	0.305
Generalized	62	10 (19.35%)	52 (80.65%)	
Control[2]				
Poor	37	5 (13.51%)	32 (87.49%)	0.765
Good	61	7(11.48%)	54 (88.42%)	
Therapy (Number)				
Monotherapy	55	4 (7.27%)	51 (92.73%)	0.439
Polytherapy	43	8 (18.6%)	35 (82.4%)	
Enzyme inducing[3]				
Non-enzyme	13	2 (15.38%)	11 (84.62%)	0.554
Enzyme	42	4 (9.52%)	38 (91.48%)	

1. Classification according to ILAE 2017
2. Good control defined as less or equal to one seizure in 6 months
3. Non enzyme inducing drug was sodium valproate while the rest were enzyme inducing.

Table 4.5: Univariate test of association between clinical factors and elevated alkaline phosphatase

Parameter	**Participants (n=98)**	**High (n=34)**	**Normal (n=64)**	**p Value (X^2)**
Sex				
Female	43	26(60.47%)	17(39.53%)	0.485
Male	55	37(67.27%)	18 (32.83%)	
Duration				
<24 months	73	46 (63.01%)	27 (36.99%)	0.653
>24 months	25	17(68.0%)	8 (32.0%)	
Type of convulsive disorder[1]				
Focal	36	12(33.33%)	24(66.67%)	0.051
Generalized	62	22 (35.48%)	42(64.52%)	
Control[2]				
Poor	37	24 (64.86%)	13 (35.14%)	0.926
Good	61	39(63.93%)	22 (37.07%)	
Therapy				
Monotherapy	55	22 (40%)	33(60%)	0.621
Polytherapy	43	12(27.9%)	31(82.1%)	
Enzyme inducing[3]				
Non-enzyme	13	7 (53.85%)	6 (47.15%)	0.604
Enzyme	42	26(61.90%)	16 (38.1%)	

1. Classification according to ILAE 2017
2. Good control defined as less or equal to one seizure in 6 months
3. Non enzyme inducing drug was sodium valproate while the rest were enzyme inducing

CHAPTER FIVE

5.0 DISCUSSION

5.1 Demographics

Majority of our participants were male, M: F of 1.2:1. This slight male predominance has also been seen in other population studies among children with epilepsy across the world (Farghaly WM et al., 2018; Mung'ala V et al., 2008; Beilmann et al., 1999; Wanigasinghe et al., 2019). In our study population of children aged 6 months to 14 years, the mean age was 7.6 years and majority of the children were below 6 years. This is also in keeping with local and worldwide statististics on paediatric epilepsy where children less than 5 years form the majority of those diagnosed and treated for epilepsy (Mung'ala V et al., 2008; Eyong' K et al 2017; Beilmann et al., 1999).

5.2 Prevalence of derangements in calcium, phosphate and alkaline phosphatase levels

Most studies have reported hypocalcaemia among children on anti-epileptic medication, however, there is no consensus on the magnitude of the hypocalcaemia and frequency has varied from one study to another. The current study found a prevalence of 13.3% (133 per 1,000 persons) which should be understood in the background of the potential of hypocalcaemia to lower seizure threshold and potentially lead to breakthrough seizures in controlled children. A comparison of the prevalence found in the current study to studies done elsewhere potrays the variation in hypocalcaemia across studies. In India, Seth et al (2017) reported hypocalcaemia in 42.5% children with cerebral palsy and epilepsy. The high prevalence could be attributed to the differences in target population where the study in India recruited children with cerebral palsy. Children with cerebral palsy often have comorbid conditions including

immobility, dietary restrictions, and poor sunlight exposure therefore these other than the treatment could also contribute to hypocalcaemia (Hough et al, 2010). In Britain (Hunter et al., 1971) looked at 105 epileptic children aged 10 to 16 years and found hypocalcaemia in 30% and raised alkaline phosphatase activity in 24%. In another study (Richens & Rowe, 1970) looked at 160 epileptic patients in a residential center and reported that 22.5% of all the participants had hypocalcaemia. These 2 studies focused on institutionalized children who could have had other risk factors of hypocalcaemia when compared to the ambulatory participants in our study. Similarly Atmasari et al (2017) found that 22% of children on carbamazepine and valproate had hypocalcaemia which was higher compared to the current study.

In Iran, Nakhaeymoghadam et al (2018) reported a prevalence of 7.5%. Notably this study looked at ambulant patients which was similar to our study. However, the duration of treatment with antiepileptic drugs was not specified. The type of anticonvulsants and duration of treatment may influence the occurrence of hypocalcaemia (Atmasari et al, 2017); therefore, the lower prevalence of hypocalcaemia in Iran compared to our study could be partly explained by duration of treatment. In Malaysia, Yi et al (2017) reported 3% prevalence of hypocalcaemia in ambulant children on anticonvulsants for more than a year. Babayigit et al (2006) found no hypocalcaemia among ambulant children on antiepileptic medication for more than 6 months. This study was limited to ambulant patients with idiopathic epilepsy and was limited to treatment with carbamazepine, valproate or oxcarbazepine monotherapy. The selective population could account for the low prevalence. In Nigeria, there was no hypocalcaemia among 89 children aged 3 to 12 years who had been on phenobarbitone monotherapy for 6 to 12 months (Ojinnaka N & Ileoje S 1997). This study was specific

to children with generalized tonic clonic epilepsy and on phenobarbitone monotherapy which could contribute to the low prevalence.

There is growing evidence that hypophosphataemia is a manifestation of disordered bone metabolism in patients on antiepileptic medication (Pack, 2003). However, the rate of hypophosphatemia is not uniform in all studies and does not necessarily parallel hypocalcaemia. The rates reported in this study were lower than the prevalence of hypocalcemia. Akpinar et al (2018) found hypophosphataemia in only 3.6% of children on anticonvulsants which was lower that our findings. However, similar to our findings, hypophosphatemia was less prevalent than hypocalcaemia. On the contrary, Borusiak found hypophosphatemia in 25.5% compared to hypocalcaemia in 24.4%. These studies reported a higher mean age of the patients with a longer duration of drug use

In another Turkish study by Ecevit et al they found a hypophosphatemia prevalence of 50% compared to 17.6% of hypocalcaemia among children who had been on valproate monotherapy. Notably the duration of use of valproate was above 2 years. It seems that the prevalence of hypophosphatemia is higher among those children who have been on antiepileptic drugs for longer period of time.

Alkaline phosphatase is a marker of bone metabolism and its level would, as found in this study, be expected to be raised in increased bone turnover. In this study, the prevalence of elevated alkaline phosphates (ALP) was found to be 34%. Similar findings have been reported in other studies even in situations where calcium was normal (Voudris et al., 2005, Babayigit et al., 2006). Krishnamoothy (2009) found that serum alkaline phosphatase was raised as early as 90 days after starting antiepileptic medication. High prevalence of elevated serum alkaline phosphatase among children receiving anticonvulsant medications have been reported in various studies (Baer et al.,

1997). This was similar to that reported in Britain (Hunter et al., 1971) who found a prevalence of 29%, while Yi et al reported it at 38%.

This study reported three markers of calcium metabolism and found variable increment in the prevalence of all of them. However, there was no instance where all the three of them were raised which may have an implication on attempts to establish biomarkers for deranged calcium metabolism. These findings are not limited to this study but have been shown in similar studies (Voudris et al., 2005,). The implication is that several biomarkers would ideally have been measured to establish the status of bone metabolism in children on antiepileptic medication.

5.2 Clinical factors associated with Calcium Metabolism Disturbance

5.2.1 Type of Anticonvulsants Used

In this study there was no significant association found between the type of anticonvulsant medications used and calcium, phosphorous and alkaline phosphatase derangement. When these drugs were categorized as either enzyme inducing or non-enzyme inducing, still no significant association was found. These findings were surprising because some studies have found a significant association between anticonvulsant drug use and calcium derangement (Yaghini et al., 2014). These studies have mainly implicated enzyme-inducing anticonvulsant drugs (Phenobarbitone, Carbamazepine and Phenytoin) compared to non-enzyme inducing anticonvulsant drugs (sodium valproate) (Konstantinos et al., 2014; Rowe, 1970; Yaghini et al., 2014). However, other studies have reported derangement in bone metabolism in patients on non-enzyme inducing drugs like valproate (Borusiak et al; 2012, Akpinar, 2018). These findings could be explained by the multiple potential mechanisms of antiepileptic drugs induced bone metabolism derangements (Pack, 2003). The differences in the findings

in these studies and the present study can be explained by the difference in study designs. Whereas these had a larger sample size, we did a sub analysis of a part of our population therefore only a small number of participants were analysed.

5.2.2 Duration of treatment and number of Anticonvulsants Used

Majority (74.5%) of the children enrolled in this study had used anticonvulsant medication for less than 24 months. There was no statistically significant difference on calcium metabolism disturbance (p-value = 0.640) among those who were on treatment for less than 24 months versus those on 24 or more months. Atmasari et al (2017) found correlation between duration of use of carbamazepine and prevalence of hypocalcaemia but no correlation between duration of use of valproate and hypocalcaemia. The current study did not look at individual antiepileptic drugs and hypocalcaemia. Also whereas Atmasari et al (2017) used a cut-off of 23 months to classify short and long duration, which is similar to the cut-off in this study, the proportion of those on medication for over 24 months was considerably small in the current study and this could confound the results. In a study by Konstantinos 2005, they were able to demonstrate increasing levels of alkaline phosphatase when taken serially at 3, 6 and 12 months. Implying duration of use of antiepileptics was significantly associated with high alkaline phosphatase. This study was however only on those on monotherapy using carbamazepine and follow up was limited to a year, it is unclear whether these levels keep on rising after the year mark.

More than half (56.12%) of those enrolled in the present study were on monotherapy while the rest were on polytherapy. Number of medications used was not found to be significantly associated with calcium metabolism disturbance. Polytherapy has been reported to be associated with reduced bone mineral density (Petty et al., 2005).

Polytherapy and use of enzyme-inducing anticonvulsant drugs were shown to be significantly associated with reduced serum calcium levels (Tombini et al., 2018). Another study conducted in Lebanon was able to demonstrate that polypharmacy and long duration of antiepileptic drugs use were significant determinants of reduction in bone mineral density and calcium concentration. (Farhat G et al., 2002). In Egypt, Mohammed et al (2017) reported significant association between polytherapy and reduced bone mineral density. However, they did not show significant correlation between reduced bone mineral density and neither serum calcium nor phosphorus implying that there may be correlation between bone mineral density and polytherapy even where the serum calcium and phosphorus fail to show it. However, the finding of an association between polytherapy and bone metabolism derangements is not universal as shown by studies that have failed to document it (Bouriask P et al., 2012; Sherifa et al, 2004; Ecevit et al, 2004). The findings are, however, difficult to compare because of differences in methodology especially the cut-off for duration of use of drugs and type of drugs in the polytherapy group.

5.2.3 Type of convulsive disorder and control of seizures

Hypocalcemia has been associated with an increase in convulsive episodes, this is postulated to be in relation to the role of calcium in neuronal firing where hypocalcemia universally enhances neuromuscular excitability throughout the body and may lead to seizures (Pengcheng et al., 2015). Phenytoin has also been postulated to cause seizures when blood calcium is low (Ali et al., 2014). Therefore, it was thought that patients with hypocalcaemia would have higher frequency of seizures. However, this study did not find any association between control of epilepsy and biochemical derangement and this has also been demonstrated in other studies (Sherifa et al., 2004), (Oner et al.,

2004). The observation could be explained by the fact that most patients with bone metabolism derangements are asymptomatic and only become symptomatic late in the disease (Voudris, 2005).

The type of seizure the child experiences, whether focal or generalized was not associated with any of the biochemical derangements. This finding is also reported in cross sectional studies in Germany and Egypt (Borusiak et al., 2012, Sherifa et al., 2004).

5.3 Study Limitations

The main limitation of this study was that calcium, phosphate and ALP were not measured before anticonvulsant use. In addition we did not have information on the normal levels of calcium, phosphate and ALP in the general population.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

i. Slightly over a tenth of the children had hypocalcemia and hypophosphatemia while a third had increased alkaline phosphatase.

ii. Calcium metabolism disturbance was not significantly associated with the clinical factors of the epileptic children enrolled in the study.

6.2 Recommendations

i. Future studies should compare the serum level of calcium, phosphate, and alkaline phosphatase between children on antiepileptic drugs and the general population.

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APPENDICES

Appendix 1: IREC Letter

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2016/148
Approval Number: 0001722

1st September, 2016

Dr. Moriasi Gesare Leah,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
01 SEP 2016
APPROVED
P. O. Box 4606-30100 ELDORET

Dear Dr. Moriasi,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"Prevalence of Disturbance in Calcium Metabolism among Paediatric Epileptic Patients on Chronic Anticonvulsants at Moi Teaching and Referral Hospital, Eldoret".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1722*** on 1st September, 2016. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 31st August, 2017. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

Appendix 2: Test Principles

CALCIUM; Test principle

Arsenazo-III dye reacts with calcium in an acid solution to form a blue-purple complex. The color developed is measured at 660 nm and is proportional to the calcium concentration in the sample.

Methodology: Arsenazo III

PHOSPHATE; Test principle

Inorganic phosphate reacts with ammonium molybdate to form a heteropolyacid complex. The use of a surfactant eliminates the need to prepare a protein-free filtrate. The absorbance at 340 nm is directly proportional to the inorganic phosphorus level in the sample. Sample blanks must be run to correct for any non-specific absorbance in the sample.

Methodology: Phosphomolybdate.

ALKALINE PHOSPHATASE; Test principle

Alkaline phosphatase in the sample catalyzes the hydrolysis of colorless *p*-nitrophenyl phosphate (*p*-NPP) to give *p*-nitrophenol and inorganic phosphate. At the pH of the assay (alkaline), the *p*-nitrophenol is in the yellow phenoxide form. The rate of absorbance increase at 404 nm is directly proportional to the alkaline phosphatase activity in the sample. Optimized concentrations of zinc and magnesium ions are present to activate the alkaline phosphatase in the sample.

Methodology: Para-nitrophenyl Phosphate

Appendix 3: Reference Ranges

Serum calcium levels

Age	Reference ranges mmol/l
Premature	1.55-2.75
0-10 days	1.9-2.6
10 days-24 months	2.25-2.75
2-12 years	2.2-2.70
Adult	2.1-2.55
>60years	2.2-2.50

Serum phosphate levels

0.85-1.45mmol/l

Serum Alkaline phosphatase

Age	Reference ranges U/l
Male	
1-12 years	<500
12-15 years	<750
>20 years	40-150
Female	
1-12 years	<500
>15years	40- 150

Serum albumin

35-44 mg/dl

Corrected calcium for hypoalbuminemia

Corrected calcium (mg/dL) = measured total Ca (mg/dL) + 0.8 * (4.0 - serum albumin [g/dL])

Appendix 4: Drug Dosages

1 Phenobarbital –

Starting dose Under 1 yr -15-30 mg/day, 1-5yrs: 30-60 mg/day, 6-12yrs: 30-90mg/day 12 and above: 60- 90 mg/day.

Maintenance dose 2-5 mg/kg/day

2 Phenytoin

Maintenance 3-5 mg/kg/day

3 Carbamazepine

Starting dose Under 1 year. 100mg/day, 1-5yrs: 150mg/day, 6-11yrs: 200mg/day, 12 and above: 400mg/day

Maintenance dose- 10-20 mg/kg/day

4 Sodium Valproate

Starting dose- Under 1yr: 100mg/day 1-5yrs: 150mg/day 6-11yrs: 400mg/day 12yrs and above: 400-600mg/day

Maintainance dose- 10-30 mg/kg/day

5 Clonazepam

Starting dose -Under 1yr 0.125mg/day 1-5yrs: 0.25mg/day 6-15yrs: 0.5mg/day

Maintenance dose Under 1 yr 0.5-1mg/day, child 6mg/day

Appendix 5: Questionnaire

Demographics

Date: ……. Medical Record Number: ……

Serial Number…….

Age ……. Sex ☐ Male ☐Female

Clinical data

1. Type of AED, check all that apply and dosage per kg

☐ Carbamazepine ……..

☐ Phenytoin ……..

☐ Phenobarbital …….

☐ Sodium Valproate …….

☐ Other Specify ……..

2. Duration of AED use in months

Carbamazepine

Phenytoin

Phenobarbital

Sodium valproate

Other

3. Type of convulsive disorder

Partial -Simple partial☐

Complex partial☐

Secondarily generalized☐

Generalized –Absence☐

Myoclonic☐

Tonic☐

Atonic☐

Tonic clonic☐

4. How many convulsive episodes have you suffered in the last 6 months?

☐Everyday

☐Atleast once a week

☐Atleast once a month

☐Once in the last 6 months

☐None

☐Good control ☐Poor control

5. Are you taking any other medication or supplements? If so which ones?

6. Any history of fractures?

Physical exam

Height …… Weight …….. Z score……..

Ambulant ☐ yes ☐ no

Muscle weakness ☐yes ☐ no

Power grade

Laboratory results

Calcium

Phosphate

Alkaline phosphatase

Albumin (conditional on hypocalcemia)

Corrected calcium

Appendix 6: Consent Form

CONSENT FORM -English version

Investigator: My name is Dr Moriasi Leah. I am a qualified doctor, registered with the Medical Practitioners and Dentists Board of Kenya. I am currently pursuing a Masters degree in Child Health and Paediatrics at Moi University. I would like to recruit your child into my research in which we will study the prevalence of hypocalcemia and derangements in markers of calcium metabolism among children on anticonvulsants at Moi Teaching and Referral hospital.

Purpose: This study will seek to determine the prevalence of derangements in calcium metabolism among the children on chronic anticonvulsants

Procedure: All children attending the neurology clinic and whose parents consent to the study will be recruited. A questionnaire will be administered to collect demographic data and clinical information on the child's condition and the medication they are taking. A short physical exam will then be done. Lastly a blood sample will be taken and tested for calcium, phosphate and ALP levels. Results will be written in the file and any serious derangements reported to the clinician for appropriate action. There shall be no payment required as all tests done will be free.

Benefits: There will be no direct benefits of participating in this study but the results of the biochemical tests will be incorporated into the care of the child. The results of this study will provide data that will be used in improving care of children on chronic anticonvulsants.

Risks: There are no anticipated risks to the participants attributable to this study. However there may be some pain and discomfort during collection of the venous blood sample.

Confidentiality: All information obtained in this study will be treated with utmost confidentiality and shall not be divulged to any unauthorized person.

Rights to Refuse: Participation in this study is voluntary, there is freedom to refuse to take part or withdraw at any time. This will not affect care given to your child in this hospital. This study has been approved by the Institutional Research and Ethics Committee (IREC) of Moi University/Moi Teaching and Referral Hospital.

If you seek any clarification you can contact the principle investigator at 0718085150 and IREC at 053 33471 Ext.3008.

Sign or make a mark if you agree to take part in the study

Parent/Guardian: …… date……

Investigator: …….Date: ……

Appendix 7: Fomu Idhinisho

FOMU IDHINISHO

Mtafiti mkuu: Jina langu ni Daktari Leah Moriasi nimehitimu na kusajiliwa na Kenya Medical Practitioners and Dentist Board. Mimi ni mwanafunzi katika chuo kikuu cha Moi nikisomea taaluma ya udaktari wa watoto. Ningependa kushirikisha mtoto wako katika utafiti wangu unaochunguza ukosefu wa kalisiamu na tofauti ya metaboli wa kalisiamu miongoni mwa watoto wanaotumia dawa za kifafa katika Hospital Kuu ya Moi.

Madhumuni: Utafiti huu utachunguza shida za metaboli ya kalisiamu miongoni mwa watoto wanaomeza dawa za kifafa.

Utaratibu wa utafiti: Watoto wote wanaohudumiwa katika kliniki ya nyurolojia na ambao wazazi watapeana idhini watashirikishwa katika utafiti huu. Dodoso litatumika kuuliza maswali kuhusu umri, hali na dawa ambazo mtoto anatumia. Mtoto atachunguzwa na kisha sampuli za damu zitachukuliwa na kupimwa kiwango cha kalisiamu, phosphate na ALP. Majibu yataandikwa katika faili na shida zozote kuripotiwa kwa daktari kwa hatua mwafaka.

Faida ya kushiriki: Hakuna malipo yoyote kwa kushiriki katika utafiti huu. Walakini, majibu ya maabara yatatumiwa katika matibabu ya mtoto. Majibu yatakayotokana na utafiti huu yatasaidia katika kuboresha mataibabu ya watoto wanaomeza dawa za kifafa.

Madhara: Hakuna madhara yoyote yatakayohusishwa katika utafiti huu.

Usiri: Mambo ya utafiti huu yatatunzwa kwa siri na kutumika katika utafiti tu. Utambulisho wako hautawekwa bayana katika makaratasi yoyote. Makaratasi yote yatawekwa katika kabati lililofungwa na kifunguu kuwa na mtafiti mkuu.

Haki ya kukataa: Kushiriki katika utafiti huu ni kwa hiari. Unaruhusiwa kutoka katika utafiti wakati wowote bila madhara yoyote. Utafiti huu umeidhinishwa na Institutional Research and Ethics Committee (IREC) ya Chuo Kikuu cha Moi/ Hospitali Kuu ya Moi. Kama una swali lolote unaweza kuafikiana na mtafiti mkuu ukitumia nambari 0718085150 na IREC kwa nambari 053 33471 Ext.3008

Tia sahihi ama weka alama iwapo umekubali kushiriki katika utafiti huu

Mzazi/Mlezi …… Mtafiti …… Tarehe ……

Appendix 8: Assent Form

A. INFORMATION SHEET

This informed assent form is for children aged between 7 to 14 years on chronic anticonvulsants in the paediatric neurology clinic and who we are inviting to participate in the research on prevalence of derangements in calcium metabolism among children on chronic anticonvulsants.

My name is Dr. Moriasi Leah,I am a resident in Pediatrics Department, Moi University. I am carrying out a research to find the prevalence of derangements in calcium metabolism among children on chronic anticonvulsants at Moi Teaching and Referral Hospital. We want to know how many of the children on anticonvulsants have low calcium and if so are there any associated factors.

I will invite you to be part of this research study. You can choose whether you will want to participate in the study or not. This will not affect the care that you receive at the clinic. We have discussed this with your parent/guardian and they are aware that we are asking you for your permission to participate in the study.

If you agree to take part in the study, your parents will also have to give permission. Should you not want to take part in the research, you will not be forced, even if your parents have agreed.

If there are any aspects that are not clear, please feel free to ask for clarification, I will be happy to assist.

Your participation will involve allowing us to ask relevant questions and drawing a blood sample to test in the laboratory.

B. CERTIFICATE OF ASSENT

I understand that the research is about establishing the prevalence of derangement in calcium metabolism among epileptic children on chronic anticonvulsants. I understand that I will take part in an interview and then have a blood sample drawn for analysis.

I have read and understood this information (or had the information read to me). Any questions I had have been answered and I know that I can ask other questions if I have any.

I agree to take part in the study.

OR

I do not wish to take part in the study and I have not signed the assent below._____ (initials by child/minor)

Only if child assents:

Print name of child _____

Signature of child: _____

Date: _____

Day/month/year

If illiterate:

I have witnessed the accurate reading of the assent form to the child, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness (not a parent)_____ AND Thumb print of participant

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the child understands that they will take part in an interview followed by drawing of a blood sample.

I confirm that the child was given an opportunity to ask questions about the study, and all the questions asked by him/her have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this assent form has been provided to the participant.

Print Name of Researcher/person taking the assent_____

Signature of Researcher /person taking the assent _____

Date _____

Day/month/year

Copy provided to the participant _____(initialed by researcher/assistant)

Parent/Guardian has signed an informed consent ___Yes ___No _____(initialed by researcher/assistant)

Appendix 9: Fomu Ya Uradhi

A. UKURASA WA UJUMBE

Hii fomu ni ya watoto wenye umri kati ya miaka 7 hadi 14 wanaotumia dawa za kifafa na wanahudhuria kliniki ya watoto wenye magonjwa ya nyurolojia. Tunawaalika kushiriki katika utafiti unaochunguza ukosefu wa kalisiamu na tofauti ya metaboli ya kalisiamu miongoni mwa watoto wanaotumia dawa za kifafa katika Hospital Kuu ya Moi.

Jina langu ni Dkt, Moriasi Leah, mwanafunzi katika idara ya Watoto ya Moi University. Mimi ninafanya utafiti unaochunguza ukosefu wa kalisiamu na tofauti ya metaboli ya kalisiamu miongoni mwa watoto wanaotumia dawa za kifafa katika Hospital Kuu ya Moi. Tunataka kujua ni watoto wangapi wanaotumia hizi dawa wako na ukosefu wa kalisiamu na kama kuna sababu yoyote inahusikana na kalisiamu kupungua.

Tunakukaribisha kushiriki katika utafiti huu na unaweza kuchagua kama utashiriki au la. Iwapo hutakubali kushiriki katika utafiti, hakutakuwa na mabadiliko katika matibabu ambayo utapata katika kliniki. Tumejadiliana na mzazi/mlezi wako na anajua ya kwamba tunakuuliza ruhusa kukushirikisha katika utafiti huu.

Ukikubali kushiriki katika utafiti huu, wazazi wako pia watahitajika kutoa ruhusa. Iwapo hautaki kushiriki hautalazimishwa hata kama wazazi wako wamekubali. Iwapo utakubali tutakuuliza maswali, na pia tutatoa damu ambayo itapimwa katika maabara.

Iwapo kuna swali lolote unaweza uliza sasa ama baadaye. Nambari yangu ya simu 0718085150.

Pendekezo hili limekubaliwa na tume ya chuo kikuu (IREC)ambayo ni kamati ya kuhakikisha kuwa wanaoshiriki katika utafiti wanalindwa kutokana na madhara.

B. CHETI CHA URADHI

Mtafiti amenifafanulia kuhusu utafiti na jinsi nitakavyoshiriki- kwa njia ya majadiliano ya kikundi au katika mahojiano. Nimesoma habari / nimesomewa habari. Nimeuliza na kujibiwa maswali yote niliyokuwa nayo. Ninafahamu kwamba nikiwa na maswali yoyote, niko huru kuuliza. Mimi kwa hiari yangu nakubali kutoa idhini ili niweze kushiriki katika utafiti huu.

Andika jina la mtoto _____

Saini ya mtoto: _____

Tarehe:_____

siku / mwezi / mwaka

Kama hawajui kusoma na kuandika:

Nimeshuhudia kusomwa kwa usahihi fomu ya idhini ya mzazi wa kijana na mzazi amekuwa na nafasi ya kuuliza maswali. Mimi nathibitisha kwamba mzazi ametoa idhini kwa uhuru.

Andika jina la shahidi (si mzazi) _____ NA alama ya kidole ya mshiriki

Saini ya shahidi _____

Tarehe _____

Siku / mwezi / mwaka

Kauli na mtafiti / mtu kuchukua ridhaa

Nimeshuhudia kusomwa kwa fomu kwa mtoto na amepewa nafasi ya kuuliza maswali. Ninadhibitisha kuwa ridhaa imepewa kwa hiari bila kushurutishwa.

Nakala ya fomu hii ya kupata kibali umetolewa kwa mshiriki.

Andika Jina la Mtafiti _____

Saini ya Mtafiti _____

Tarehe _____

Siku / mwezi / mwaka

Nakala zimetolewa kwa mshiriki

Jina la mtafiti _____

Sahihi ya mtafiti _____