BONE METABOLISM ABNORMALITIES IN CHILDREN WITH EPILEPSY AT RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL, CAPE TOWN, SOUTH AFRICA.

By

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This minor dissertation is my own original work and it has not been presented and will not be presented to any other University for a similar or any other degree award. This work has not been reported or published prior to registration to the above mentioned degree.

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ABSTRACT:

Introduction: Epilepsy is the most common neurological condition worldwide. Literature on the antiepileptic medications and biochemical markers of bone metabolism has revealed inconsistent results. Most of these studies were undertaken in Europe and America where the burden and the associated comorbidities are different to the ones in Africa.

Methods: A hospital based case control study was undertaken at Red Cross War Memorial Children's Hospital where children were recruited from a dedicated Epilepsy clinic and controls were obtained from a day surgical ward. Blood and urine samples were taken for the assessment of markers of bone metabolism.

Results: Seventy-five cases and 75 controls were recruited**.** The median age for the children with epilepsy was 9 years with a range of 1 to 17 and controls 3 years with a range of 1 to 12. Vitamin D deficiency was present in 11(16.2%) of children with epilepsy compared to 6(8.8%) in the control group. Vitamin D insufficiency was present in 30(44.1%) in children with epilepsy compared to 27(39.7%) in the control group. Children with epilepsy on enzyme inducing AEDs had lower mean Vitamin D levels (24.67±11.4 vs 30.72±7.4, p=0.08), lower mean serum phosphate levels (1.39±0.2 vs 1.76±0.7,p=0.000) and a higher mean parathyroid hormone levels (4.47±2.33 vs 2.7±0.97, p=0.03) compared to the control group. Children with Epilepsy on enzyme inhibitors had higher mean Vitamin D2 (0.44±0.37 vs 0.25±0.07,p=0.000004) and mean Vitamin D3 (2.26±0.86 vs 1.61±1.06,p=0.028) compared to children on enzyme inducers Dietary intake and ancestry did not influence Vitamin D levels between the cases and controls.

Conclusion and Recommendations: Vitamin D deficiency is common in children with epilepsy on AEDs. Children on enzyme inducing AEDs should be investigated for vitamin D deficiency and managed accordingly.

1.0 Introduction

Epilepsy is one of the common neurological disorders with an estimated 50 million people affected worldwide, 85% being in the low and middle income countries (Ngugi et al., 2010; "WHO. Epilepsy in the WHO Africa region, Bridging the Gap: The Global campaign against epilepsy '"Out of the Shadows."'; 2004," n.d.). In sub-Saharan Africa more than 50% of people with epilepsy are children with 69% of convulsive seizures beginning in childhood (Kariuki et al., 2014). Most children with epilepsy are prescribed one or more antiepileptic drug (AED) for the control of seizures. However, in sub-Saharan Africa only 36% have access to AEDs with 95% of this group managed with phenobarbitone (Kariuki et al., 2014). AEDs are associated with acute and chronic side effects. One of the chronic side effects of AED use is abnormalities in bone metabolism. Several studies have shown that long term use of AEDs lead to abnormalities in bone health (Farhat et al., 2002; Pack and Walczak, 2008; Sheth et al., 2008).

1.1 Bone Metabolism:

The structure of the bone is composed of organic and inorganic components which are continuously remodeled throughout life. Bone formation is initiated by osteoblasts, and osteoclasts are involved in bone resorption while bone mechanical stresses are monitored by osteocytes (*Braunwald E, Harrison TR. Harrison's principles of internal medicine. 15th ed. New York: McGraw-Hill, Medical Pub. Division; 2001*, n.d.). Bone remodeling is monitored by assessing several biochemical markers which are grouped into markers of bone deposition and bone resorption (*Braunwald E, Harrison TR. Harrison's principles of internal medicine. 15th ed. New York: McGraw-Hill, Medical Pub. Division; 2001*, n.d.). Elevated levels of bone specific alkaline phosphatase and osteocalcin serve as markers of bone deposition since they are raised with increased osteoblast activity (*Braunwald E, Harrison TR. Harrison's principles of internal medicine. 15th ed. New York: McGraw-Hill, Medical Pub. Division; 2001*, n.d.). The markers of bone deposition and resorption are derived from osteoblast and osteoclast activity respectively. The biochemical markers for bone resorption are urinary hydroxylysine, hydroxyproline and hydroxypiridium collagen-cross links. The biochemical markers of bone metabolism are physiologically raised during period's growth spurts and bone repair. They reflect the physiological state of bone remodeling at a given time (*Braunwald E, Harrison TR. Harrison's principles of internal medicine. 15th ed. New York: McGraw-Hill, Medical Pub. Division; 2001*, n.d.).

Physical activity, calcium intake and nutrition also affect the rate of bone remodeling. Bone remodeling is also regulated by other growth factors and hormones such as Parathyroid hormone, Vitamin D_3, estrogens, androgens, insulin like growth factors I and II and Tumour necrosis factor. Together with Parathyroid hormone, Vitamin D regulates osteoblastic and osteoclastic activity thus maintaining Calcium and Phosphate homeostasis. As a result of this role Vitamin D is vital for the maintenance of bone mineral density (*Braunwald E, Harrison TR. Harrison's*

principles of internal medicine. 15th ed. New York: McGraw-Hill, Medical Pub. Division; 2001, n.d.).

1.2 Vitamin D

Vitamin D is a fat soluble vitamin which is essential for Calcium metabolism. The main source of Vitamin D is ultraviolet radiation which accounts for about 90% of the body daily requirement. Sufficient Vitamin D can be produced in fair skin individuals following a 10 to 15 minutes of sun exposure between 10am and 3pm in summer, spring and autumn. Longer duration of sun exposure will be required for individuals of African descent (Rovner and O'Brien, 2008). Other forms of Vitamin D, ergocalciferol (Vitamin D_2) and cholecalciferol (Vitamin D_3) are derived from plants and animals respectively (Norman, 1998). The epidermis of the skin has the highest concentration of the 7-dehydrocholesterol. Following sun exposure 7-dehydrocholesterol is converted to cholesterol and transported to the liver bound with vitamin D binding protein. Vitamin D undergoes hydroxylation in the liver to 25-hydroxycholecalciferol. The 25-hydroxycalciferol is then transported to the kidney bound to vitamin D binding protein where it undergoes second hydroxylation to 1, 25-dihydroxycholecalciferol, the active form of vitamin D (Holick, 2004; Holick et al., 1995; Norman, 1998). Vitamin D is paramount for maintaining bone health by promoting intestinal absorption of calcium and its bone deposition (Holick, 2006; Misra et al., 2008).

1.3 Normal Ranges of Blood Vitamin D Levels

The best indicator of the total body vitamin D level is the circulating 25-hydroxycholecalciferol. Its half-life is 2 to 3 weeks compared to 4 hours of the active form, 1, 25 dihydroxycholecalciferol (Misra et al., 2008). The normal value of Vitamin D is more than 30 ng/ml, insufficiency 20-30ng/ml and deficiency less than 20 ng/ml (Fong and Riney, 2014).

The effect of antiepileptic medications (AEDs) on Vitamin D metabolism and bone health has been an area of great interest for clinicians for more than 3 decades (Hahn et al., 1972; Offermann et al., 1979; Richens and Rowe, 1970). Studies have suggested AEDs induce hepatic cytochrome P450 leading to the catabolism of Vitamin D, causing reduction of calcium absorption from the intestine, hyperparathyroidism and increased calcium demineralization from the bones (Pascussi et al., 2005; Shellhaas et al., 2010; Zhou et al., 2006).

1.4 Vitamin D and Antiepileptic medication

Compared to the healthy controls, abnormalities in bone metabolism and an increased risk of fractures are more common in children with epilepsy on antiepileptic medication (El-Hajj Fuleihan et al., 2008; Gniatkowska-Nowakowska, 2010; Souverein et al., 2005). The fracture risk has been demonstrated to be higher for hepatic enzyme inducing AEDs such as carbamazepine, phenobarbitone and phenytoin than the non-inducers. Sodium valproate,

carbamazepine, phenobarbitone and clonazepam have also been shown to have a dose response relationship to the risk of fractures (Vestergaard et al., 2004).

Hypocalcaemia, hypophosphataemia, low Vitamin D levels and elevated parathyroid hormone levels have been shown in several studies to be associated with the use of AEDs. The effect is more with AEDs known to have cytochrome P450 enzyme inducing activity (Ali et al., 2004; Erbayat Altay et al., 2000; Pack et al., 2004; Sheth, 2004). The mechanism suggested for the low levels of calcium and phosphate is the increased cytochrome P450 activity by AEDs which augments the catabolism of Vitamin D (Pack et al., 2004). Despite the plausible logic behind enzyme induction and AEDs, the data has been inconsistent with several other studies failing to demonstrate low levels of calcium and phosphate with enzyme inducing AEDs (Kulak et al., 2004; Tsukahara et al., 2002).

Patients with epilepsy who are prescribed AEDs have elevated levels of parathyroid hormone. This effect is postulated to be secondary to the low serum vitamin D levels in these patients (Välimäki et al., 1994; Weinstein et al., 1984). The rate of bone turnover is elevated with increased levels of parathyroid hormone hence reducing the bone mineral density (Weinstein et al., 1984).

The use of AEDs is associated with low levels of 25-hydroxycholecalciferol. In an adult cross sectional study of 58 patients in Brazil on one or two AEDs, 25-hydroxycholecalciferol levels were significantly lower in the AEDs group as compared to the control group. Thirty four percent of the AEDs group had levels of 25-hydroxycholecalciferol levels below 20ng/dl as compared to 7% in the control group (Kulak et al., 2004). In this Brazilian study the alkaline phosphatase levels were found to be significantly lower in the AEDs group as compared to the control group (Kulak et al., 2004). Similarly in another phamarcoepidemiologic case control study in Denmark, more than 50% of the cases one or more AEDs had low levels of vitamin D as compared to controls (Vestergaard et al., 2004).

In an American cross sectional study including children 3-17 years, 25-hydroxycholecalciferol levels less than 25ng/dl were significantly associated with partial seizures, female sex and increasing body mass index (Shellhaas et al., 2010). In this study 75% of the children had insufficient vitamin D levels (Shellhaas et al., 2010).

1.5 Vitamin D and Antiepileptic Monotherapy (Table 1)

Carbamazepine is a widely used first line medication for focal and generalized seizures in children. Carbamazepine is associated with abnormalities of Vitamin D metabolism. In a cross sectional and retrospective study in Turkey, the levels of vitamin D were significantly lower in children on carbamazepine as compared to the control group (Kumandas et al., 2006). Similar findings were observed in a longitudinal prospective study in Korea (Kim et al., 2007). However other studies in patients with Epilepsy on Carbamazepine did not demonstrate similar findings (Erbayat Altay et al., 2000; Samaniego and Sheth, 2007).

Phenobarbitone a widely used AED in low income countries has shown inconsistent findings with abnormalities of bone metabolism. The level of 25-hydroxycholecalciferol is reduced among patients with epilepsy on Phenobarbitone compared to controls (Farhat et al., 2002; Vestergaard et al., 2004). Phenobarbitone is known to cause induction of hepatic cytochrome P450 thus increasing the catabolism of Vitamin D (Verrotti et al., 2010). This effect causes hypocalcaemia by reducing intestinal calcium absorption leading to hyperparathyroidism and bone demineralization (Verrotti et al., 2010). However other studies on phenobarbitone failed to show similar results (Gissel et al., 2007; Weinstein et al., 1984).

Valproic acid is suggested to stimulate osteoclast activity thus leading to bone demineralization (Valsamis et al., 2006). Data on valproic acid and markers of bone metabolism is inconsistent (Oner et al., 2004; Sheth et al., 1995). Studies in Turkey and Japan showed levels of calcium concentration in children on valproic acid to be low (Tekgul et al., 2006; Tsukahara et al., 2002). However, other studies in children on valproic acid had normal levels of calcium and phosphorous (Kim et al., 2007; Kumandas et al., 2006). Similarly studies on valproic acid and serum 25-hydroxycholecalciferol revealed inconsistent findings. Studies by Tekgul *et al* and Nicolaidou *et al* showed that children on valproic acid had low levels of 25-hydroxycholecalciferol compared to controls (Nicolaidou et al., 2006; Tekgul et al., 2006). Nevertheless other studies in Japan, Turkey and Germany had normal levels of 25-hydroxycholecalciferol with valproic acid (Babayigit et al., 2006; Tsukahara et al., 2002).

Studies on lamotrigine and markers of bone metabolism in children are scarce. Studies in adults on lamotrigine had shown the serum levels of calcium, 25-hydroxycholecalciferol and parathyroid hormone were within normal limits (Pack et al., 2005; Stephen et al., 1999).

An adult study of premenopausal women with epilepsy in Korea had shown that topiramate may have long term effects on bone metabolism. In this matched case control study the level of serum calcium, bone specific alkaline phosphatase and osteocalcin were lower compared to controls (Heo et al., 2011).

1.6 Vitamin D and Multiple AEDs (Table 1)

Multiple AEDs have also been associated with abnormalities in bone metabolism. A Canadian study involving children 3-17 years had showed low body height percentile and low osteocalcin in children on valproate and lamotrigine compared to children on either medication (Guo et al., 2001). Another study in Germany including children 4-12 years, the mean 25-hydroxycholecalciferol were significantly lower in children on multiple AEDs compared to children on monotherapy (Nettekoven et al., 2008). Bergqvist *et al* had shown in a study in USA that a decrease of 7ng/dl of serum 25-hydroxycholecalciferol occurred with additional of each AED in children with intractable epilepsy (Bergqvist et al., 2007). In this study 66% of the children with intractable epilepsy were vitamin D insufficient with calcium and phosphorous

within the normal range (Bergqvist et al., 2007). Another study in Lebanon the level of 25-hydroxycholecalciferol was found to be less than 20ng/dl in 57% of the patients on multiple AEDs (El-Hajj Fuleihan et al., 2008).

1.7 Vitamin D and Bone Mineral Density (Table 1)

In a prospective study in Turkey by Babayigit *et al* involving 68 children with Epilepsy and 30 controls, with a mean age 12.2 and 13.09 respectively showed bone mineral density was lower in children taking valproic acid, carbamazepine and oxcarbazepine as compared to controls (Babayigit et al., 2006). A cross sectional study in Lebanon including 71 patients (42 adults and 29 children/adolescents) showed generalized seizures, polypharmacy and duration of epilepsy were significant predictors of bone mineral density (Farhat et al., 2002). The serum vitamin D level has been shown in different studies not to correlate with bone mineral density (El-Hajj Fuleihan et al., 2008; Farhat et al., 2002). However another study showed a correlation between vitamin D and bone mineral density. In an adult prospective study including drug naïve patients the serum level of vitamin D was significantly lower in patients taking carbamazepine 6 months after initiation of treatment (Kim et al., 2007). In this study the low level of vitamin D correlated with decreased bone mineral density. A similar effect was not observed with valproic acid and lamotrigine (Kim et al., 2007).

Table 1: Summary of studies showing inconsistent findings on Vitamin D level and AED by year of publication

Country	Author	AED Studied	Study Design	Sample size	Main objective	Key Findings
Germany	Nettekoven *et al* (2008)(Nettekoven et al., 2008)	CBZ,VPA,LVT, LMT,TMP,OXZ	Cross sectional	38 Children Mean 8.4±1.7	Effect of AED on Vit D and markers of bone turnover	AED was associated with low Vit D
Lebanon	Fuleihan *et al* (2008)(El-Hajj Fuleihan et al., 2008)	PHT, PT, CBZ, VPA, LMT, CNZ	Longitudinal	106 Adults (18-60) 88 Children (10-17)	Predictors of BMD	Low Vit D levels, BMD was decreased in adults
USA	Bergqvist *et al* (2007)(Bergqvist et al., 2007)	Newer AED and KD	Longitudinal study	45 Children Mean 5.1±2.7	Vit D status in children with intractable epilepsy on AED and KD	Newer AED and KD associated with low Vit D
South Korea	Kim SH *et al* (2007)(Kim et al., 2007)	CBZ, VPA, LMT	Longitudinal study	33 Adults (18-50)	Effect of AED monotherapy on bone biochemical markers and BMD	CBZ associated with a decrease in BMD and Vit D
Turkey	Babayigit *et al* (2006)(Babayigit et al., 2006)	CBZ, VPA, OXZ	Longitudinal study	68 children	Effect of AED on biochemical markers of bone metabolism and BMD	Normal Vit D levels but decreased BMD
Turkey	Tekgul *et al* (2006)(Tekgul et al., 2006)	CBZ, VPA, PHT	Longitudinal study	30 Children	Effect of AED monotherapy on bone mineral status	AED monotherapy was associated with low Vit D
Israel	Kumandas S *et al* (2006)(Kumandas et al., 2006)	CBZ, VPA	Cross sectional Retrospective	33 Children	Effect of AED on BMD	Vit D and BMD lower in the AED group compared to healthy children
Greece	Nicolaidou *et al* (2006)(Nicolaidou et al., 2006)	CBZ, VPA	Longitudinal study	51 children	Effect of AED on Biochemical markers of bone metabolism	AEDs associated with low Vit D
USA	Pack *et al* (2005)(Pack et	CBZ, LMT, PT, VPA	Longitudinal study	93 premenopau	Effects of AED monotherapy on bone mineral status and BMD	Lower Vit D and BMD associated with PT

	al., 2005)			sal women		
Denmark	Vestergaard P *et al* (2004)(Vestergaard et al., 2004)	PHT,CBZ,OXZ, CNZ, LMT,VPA,TG,PT,PMD,TMP,VG	Case Control	124655 fracture cases 373962 controls	Fracture risk associated with AED	Limited increase fracture risk with AED
Lebanon	Farhat G *et al* (2002)(Farhat et al., 2002)	PHT, CBZ, PT, VPA, LMT,CNZ, GBT,ETX,TMP	Cross sectional	42 Adults and 29 children	Effect AED on Vit D levels and bone density Effect of single and multiple drugs	Low Vit D levels in over 50% of patients. Generalized seizures and polypharmacy were predictors of BMD
Japan	Tsukahara *et al* (2002)(Tsukahara et al., 2002)	CBZ, VPA, PHT, ETX, CNZ	Longitudinal study	18 children (5-16)	Effect of AED on bone mineral status and BMD	Normal Vit D levels but decreased BMD
Canada	Guo *et al* (2001)(Guo et al., 2001)	LMT, VPA	Longitudinal study	53 Children (3-17)	Long term effects of VPA and LMT on bone metabolism	Normal Vit D levels
Turkey	Erbayat E *et al* (2000)(Erbayat Altay et al., 2000)	CBZ, VPA	Cross Sectional	36 Children	Effect of AED monotherapy on bone biochemical markers and BMD	AED had no effect on bone biochemical markers or Vit D
Scotland	Stephen *et al* (1999)(Stephen et al., 1999)	PHT, CBZ, PT, VPA, LMT, GBT, TMP, VG	Case Control	Adults	Effect of AED on bone mineral status and BMD	AED was associated with decreased BMD but normal Vit D
USA	Weinstein RS *et al* (1984)(Weinstein et al., 1984)	PHT, CBZ, PT, VPA, CNZ	Cross sectional	120 Adults	Effect of AED on ionized Ca and Vit D	Low ionized Ca in patients on AED but normal Vit D level

PHT: Phenobarbital CBZ: Carbamazepine PT: Phenytoin VPA: Valproic acid LMT: Lamotrigine CNZ: Clonazepam
GBT: Gabapentin ETX: Ethosuximide TMP: Topiramate OXZ: Oxcarbazepine PMD: Primidone TG: Tiagabine
LVT: Leviteracetam VG: Vigabatrin AED: Antiepileptic Drug KD: Ketogenic Diet

2.0 PROBLEM STATEMENT

Literature on the Vitamin D metabolism and antiepileptic medication in individuals with epilepsy has revealed inconsistent results (Table 1). Most of these studies were undertaken in Europe and North America in patients who are well controlled on monotherapy.

About 100 patients are seen at the epilepsy clinic at Red Cross War Memorial Children's Hospital every month. Most of these patients have difficult to control epilepsy and are on at least two AEDs. It is not a routine practice to supplement patients with epilepsy on AEDs with Vitamin D at Red Cross War Memorial Children's Hospital. Data on the spectrum of abnormalities of bone metabolism in children with epilepsy at Red Cross War Memorial Children's Hospital in not known. As a result there is no evidence to suggest whether these children are Vitamin D deficient and thus require routine supplementation with vitamin D and calcium.

3.0 RATIONALE

The Epilepsy clinic at Red Cross War Memorial Children's Hospital attends to children with difficult to treat Epilepsy. Most of these children are on at least two AEDs which may have an effect on the bone metabolism.

This study will provide important data on the effect of single or multiple AEDs in children with epilepsy on bone metabolism in an African setting. From this local protocols may be developed on whether children on AEDs should be routinely supplemented with vitamin D and calcium, based on the results of this study.

4.0 STUDY HYPOTHESIS

4.1 Null Hypothesis:

The use of AEDs in children with Epilepsy is not associated with abnormalities of bone metabolism.

4.2 Alternative Hypothesis:

The use of AEDs in children with epilepsy is associated with abnormalities of bone metabolism.

5.0 OBJECTIVES

5.1Broad Objective: To determine the abnormalities of bone metabolism in children with Epilepsy attending Epilepsy clinic at Red Cross War Memorial Children's Hospital in Cape Town, South Africa.

5.2 Specific Objectives:

1. To determine the proportion of Vitamin D insufficiency in children on antiepileptic drugs attending Epilepsy clinic at Red Cross War Memorial Children's Hospital.
2. To determine the biochemical markers of bone metabolism (serum level of calcium, phosphorous, parathyroid hormone, urinary deoxypyridinoline and urinary creatinine) in children on antiepileptic drugs attending Epilepsy clinic at Red Cross War Memorial Children's Hospital.
3. To determine the predictors of vitamin D insufficiency in children on antiepileptic drugs attending Epilepsy clinic at Red Cross War Memorial Children's Hospital.

6.0 METHODOLOGY

6.1 Study Design: This was unmatched case control study.

6.2 Study Site: The study was conducted at the Epilepsy clinic at the Red Cross War Memorial Children's Hospital in Cape Town, South Africa. This is the University affiliated tertiary referral centre attending children with Epilepsy across the spectrum from those who require primary care to those with complex quaternary needs. The hospital runs an Epilepsy clinic every Wednesday where on average 100 patients are seen every month.

6.3 Study Population: All patients less than 18 years of age with Epilepsy attending Epilepsy clinic at the Red Cross War Memorial Children's Hospital.

6.4 Study Duration: The study data was collected from July 2014 to May 2015. Analysis of the collected samples and group demographics followed over the next 18 months.

6.5 Inclusion Criteria:

Cases:

i. All children with Epilepsy attending Epilepsy clinic at Red Cross War Memorial Children's Hospital who were on a single or multiple antiepileptic drug for at least 3 months' duration, which were noted to be associated with influences on bone metabolism (phenobarbitone, carbamazepine and sodium valproate) at stable dosage, i.e. not increasing or reducing
ii. The second group was children with Epilepsy but not on known prescribed enzyme inducing AEDs (phenobarbitone, carbamazepine and phenytoin) or on polytherapy.
iii. All children whose parents' consented to participate in the study.

Controls:

i. The controls were obtained from the patients who are coming for surgical day outpatient procedures at Red Cross War Memorial Children's Hospital. These patients have routine bloods and intravenous line insertion for their surgical procedures. Consent was requested for 3 mls of blood to be used for this study.

6.6 Exclusion Criteria:

i. All children suspected to have a condition which affects bone metabolism, eg: Non ambulatory (inappropriate for developmental age), renal diseases, hyperparathyroidism, gastrointestinal disorders, diabetes mellitus, cerebral palsy, hemiplegia and liver insufficiency.
ii. All children on oral corticosteroids.
iii. All children on Calcium or Vitamin D supplementation.

6.7 Sample size calculation:

The sample size was calculated using the following formula.

$$n_1 = \frac{(Z_\alpha + Z_\beta)^2 pq(r+1)}{r\,(p - p_2)^2}$$

$$n_2 = rn_1$$

$$p = \frac{p_1 + rp_2}{r+1}$$

$$q = 1 - p$$

Where by
n_1 = Number of cases
n_2= Number of controls
Z_α=critical value for two-sided confidence level (confidence interval) 95%= 1.96
Z_β= standard normal deviate for one-tailed test based on beta level 0.84 for power of 80%
r = Ratio of Cases to Controls is 1:1
p_1 = Proportion of cases with Vitamin D deficiency = 22% (Fong and Riney, 2014).
p_2 = Estimated Proportion of controls with Vitamin d deficiency = 5%

The sample size calculated was 63. The sample size was increased by 10% to make allowance for incomplete data, non-responses and other factors that may decrease the yield of responses. Thus, the minimum sample size was 70 for the cases. The control group will have 70 patients to make up a ratio of 1:1 for cases and controls.

6.8 Sampling Procedure:

All children who met the inclusion were consecutively enrolled until the sample size was reached. Cases were recruited from the Epilepsy clinic every Wednesday and controls from the surgical day outpatient ward. Patients were recruited throughout the year to include the winter and summer seasons.

6.9 Data Collection:

All information was collected using a standardized questionnaire specifically designed for the purpose of the study. The data collected were:

- Demographic details: Age, sex, weight, height, span, ancestry etc
- Dietary intake
- Seizure onset and the types of seizures
- Types of antiepileptic drugs used, age at onset of medication, duration etc
- Antiepileptic drug levels
- Investigation: A blood sample was taken for 25-hydroxycholecalciferol, serum calcium, phosphate and parathyroid hormone.

- A urinary sample was collected for assessment of urinary deoxypyridinoline and creatinine.

6.10 Investigations: A 3 mls of blood specimen was obtained from the cases and controls for the measurement of 25-hydroxycholecalciferol, serum calcium, phosphate and parathyroid hormone levels.

6.11 Serum Vitamin D: The serum vitamin D was determined by using ADVIA centaur assay. The assay is an antibody competitive immunoassay that uses an anti-fluorescein monoclonal mouse antibody covalently bound to paramagnetic particles (PMP), an anti-25(OH) vitamin D monoclonal mouse antibody labeled with acridinium ester (AE), and a vitamin D analog labeled with fluorescein.
The serum vitamin D level was classified as follows(Fong and Riney, 2014)
Vitamin D deficient < 20 ng/ml
Vitamin D insufficient 20-30 ng/ml
Normal > 30ng/ml

6.12 Serum Parathyroid hormone: The parathyroid hormone was determined using the Cobas 2012 machine. The assay was done using the sandwich principle.
The parathyroid hormone was classified as follows
Normal 1.6 – 6.9 pmol/L

6.13 Serum Calcium: The serum calcium was measured using the Beckman Coulter Synchron Systems 2007. A precise volume of sample 40µL was mixed with the buffered solution. The calcium concentration was determined by indirect potentiometry utilizing a calcium ion selective electrode in conjunction with a sodium reference electrode.
The serum calcium level was classified as follows
Normal Serum calcium 2.23 – 2.58 mmol/L
Hypocalcaemia < 2.23 mmol/L
Hypercalcaemia >2.58 mmol/L

6.14 Serum Phosphate: The serum phosphate was determined using Beckman Coulter Synchron 2007. The phosphorous reagent was used to measure the phosphorous concentration by a timed endpoint method. The machine automatically proportions the appropriate sample and reagent volumes into a cuvette.
The serum phosphate level was classified as follows
Normal 0.81 – 1.49 mmol/L
Hypophosphataemia < 0.81 mmol/L
Hyperphosphataemia > 1.49 mmol/L

6.15 Alanine Amino Transferase: The Alanine amino transferase (ALT) was determined using the Beckman Coulter Synchron 2007. The ALT reagent was used to measure analyte activity by a kinetic rate method. The machine was automatically mix the appropriate sample and reagent volumes into the cuvette.
The ALT level was classified as follows.
Normal 14 – 54 IU/L

6.16 Aspartate Amino Transferase: The Aspartate amino transferase (AST) was determined using the Beckman Coulter Synchron 2007. The AST reagent was used to measure AST activity using the enzymatic rate method. The machine automatically mixes appropriate sample and reagent volumes into a cuvette.
The AST level was classified as follows.
Normal 15 – 41 IU/L

6.17 Urinary Deoxypyridinoline: The urinary deoxypyridinoline (DPD) was determined using the Siemans Immulite 2000. The urinary sample and the reagent was incubated into the machine for 30 minutes.
The urinary DPD was classified as follows
Normal urinary DPD Female 3.0 – 7.4 nM DPD/mmol creatinine
Male 2.3 – 5.4 nM DPD/mmol Creatinine

6.18 Dietary Assessment: Dietary assessment to assess the intake of the main dietary sources of vitamin D was done by the dietician Shihaam Cader. The food sources was assessed include fish, beef, eggs, cheese and cod liver oil. The frequency and amount of intake of these food sources was estimated using the specific questionnaire designed for this study.

6.19 Ethical Clearance:

The ethical clearance to conduct the study was obtained from the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee.(HREC REF: 560/2014)

6.20 Ethical Considerations:

Prior to enrolment into the study, a written informed consent and assent was obtained from parents/guardians whose children have Epilepsy and for the controls. Assent unless precluded by cognitive impairment was obtained in children of eight years and above. The informed consent was translated into the main local languages in Cape Town, Xhosa and Afrikaans. The aims, investigations and benefits of participating in the study was explained to the parents/guardians whose children were enrolled. The children may experience pain during blood taking but this were minimized with the use of emla to numb the skin before taking blood. Additional information requested by the parents/guardians were provided by the investigator.

Children enrolled into the study were identified by unique study numbers. Names of the patients and their places of residences were not included in the computerized database and all data were handled in the strictest of confidence. Laboratory results were provided to the attending clinicians and explained to the parents/guardians to facilitate appropriate management of the patients. Patients with abnormal results were supplemented with Vitamin D. Children in the control group with abnormal results were contacted and reviewed by the co-investigating Paediatric endocrinologist for appropriate management.

6.22 Data Processing and Analysis

All the questionnaires were filled in by the investigator. Data entry, cleaning and analysis were done using SPSS (Statistical Package for Social Science) software version 18.

The data were summarized using frequency tables. Children with Vitamin D insufficiency were summarized using proportions. Analyses were done using chi-squared or fishers exact test where necessary.

Serum levels of calcium, Vitamin D, phosphate, parathyroid hormone and urinary deoxypyridinoline and creatinine were summarized using mean and standard deviation or median and range where the data was skewed. Analysis was done using student t test or Mann Whitney test where necessary.

A p value of less than 0.05 was considered as evidence of statistically significant association or difference.

7.0 RESULTS:

During the study period between July 2014 and May 2015, 150 children met the inclusion criteria and were recruited into the study. Seventy-six children were of mixed ancestry, 60 African and 14 caucasians. Seventy-five cases and 75 controls were recruited. The median age for the children with epilepsy was 9 years with a range of 1 to 17 and controls 3 years with a range of 1 to 12. The male to female ratio for the children with epilepsy was 1.3:1 and controls 4:1. The majority of the controls were boys because the most common cause of admission for the day surgical ward is circumcision.

7.1 Vitamin D Deficiency.

The proportion of children with epilepsy on AEDs with Vitamin D level less than 20ng/ml was 16.2% compared to 8.8% in the control group but this was not statistically significant. Only 68 cases and 68 controls hade adequate samples for the Vitamin D studies. (Table 1)

Table 1: Proportion of Vitamin D deficiency in children with Epilepsy compared to the control group

Vitamin D Level (ng/ml)	Patients with Epilepsy		Controls Group		Total	P value
	No	%	No	%		
Less than 20	11	16.2	6	8.8	17	**0.264**
20 to 30	30	44.1	27	39.7	57	
Greater than 30	27	39.7	35	51.5	62	
Total	68	100	68	100	136	

7.2 Vitamin D Parameters

The vitamin D2 and D3 were significantly lower in patients with epilepsy on Cytochrome P450 inducers compared to those on Cytochrome P450 inhibitors and controls (Table 2)

Table 2: Comparison Vitamin D2, D3 and Parathyroid Hormone in children with Epilepsy on cytochrome P450 inducers, inhibitors and controls

	Cytochrome P450 Inducers n=14	Cytochrome P450 Inhibitors n=48	P value	Cytochrome P450 Inducers n=14	Controls n=75	P value
Mean Vitamin D	24.67±11.4	27.96±7.7	0.23	24.67±11.4	30.72±7.4	**0.08**
Mean Vitamin D2	0.25±0.07	0.44±0.37	**0.000004**	0.25±0.07	0.4±0.17	**0.0018**
Mean Vitamin D3	1.61±1.06	2.26±0.86	**0.028**	1.61±1.06	2.58±0.86	**0.004**
Corrected Serum Calcium	2.29±0.06	2.36±0.08	**0.009**	2.29±0.06	2.33±0.12	0.15
Serum Phosphate	1.41±0.23	1.38±0.25	0.7	1.41±0.23	1.77±0.7	**0.0015**
Parathyroid Hormone	4.47±2.33	2.64±0.99	**0.02**	4.47±2.33	2.7±0.97	**0.03**

7.3 Biochemical Markers of bone metabolism

The mean Vitamin D, $24,25(OH)_2D_3$, serum calcium, serum phosphate and urinary DPD/creatinine ratio were all significantly lower in children with epilepsy compared to the control group. (Table 3)

Table 3: Biochemical parameters of children with Epilepsy compared to the control group

	Patients with Epilepsy	Control Group	p-value
Vitamin D (ng/ml)	**N=68**	**N=68**	
Mean±SD	27.56±8.6	30.30±7.4	**0.024**
24,25(OH)$_2$D$_3$ (ng/ml)	**N=68**	**N=68**	
Mean±SD	2.14±0.9	2.58±1.1	**0.014**
25(OH)D$_2$ (ng/ml)	**N=68**	**N=68**	
Mean±SD	0.39±0.3	0.4±0.1	0.78
25(OH)D$_3$/24,25(OH)2D3	**N=68**	**N=68**	
Mean±SD	14.17±4.3	13.15±3.9	0.152
25(OH)D$_3$/D$_3$	**N=68**	**N=68**	
Mean±SD	32.71±2.4	34.26±2.5	0.715
Serum Calcium	**N=73**	**N=69**	
Mean±SD	2.38±0.09	2.34±0.12	**0.026**
Calcium Corrected	**N=71**	**N=68**	
Mean±SD	2.34±0.08	2.32±0.12	0.553
Serum Phosphate	**N=72**	**N=68**	
Mean±SD	1.39±0.2	1.76±0.7	**0.000**
Parathyroid Hormone	**N=68**	**N=52**	
Mean±SD	3±1.51	2.7±0.97	0.187
Urinary DPD	**N=38**	**N=39**	
Mean±SD	164±107	185±94	0.363
Urinary Creatinine	**N=39**	**N=43**	
Mean±SD	11.57±21.9	5.47±3.26	0.094
DPD/Creatinine ratio	**N=38**	**N=39**	
Mean±SD	21.8±10.25	41.6±25.02	**0.000**

7.4 Predictors of Vitamin D Deficiency

Type of AEDs, number of AEDs, seizure semiology and whether a child had a known epilepsy syndrome or not, were not associated with Vitamin D deficiency. (Table 4)

Table 4: Clinical Parameters and Vitamin D levels in children with epilepsy Vitamin D Level

	Less than 20ng/ml		Greater than 20ng/ml		P value
	No	%	No	%	
Enzyme Inducers					
Yes (n=24)	6	54.5	18	31.6	0.144
No (n=44)	5	45.6	39	68.4	
No of AEDs used					
1-2 (n=52)	9	81.8	43	75.4	0.648
>3 n=16)	2	18.2	14	14.6	
Seizure Semiology					
Focal (n=39)	7	63.6	32	56.1	0.645
Generalized (n=29)	4	36.4	25	23.9	
Epilepsy Syndrome					
Yes (n=18)	3	27.3	15	26.3	1.0
No (n=50)	8	72.7	42	73.7	

7.5 Dietary Intake

There was no difference in the dietary intake between the children with epilepsy on AEDs and controls.

7.6. Ancestry

Among children with epilepsy, the mean vitamin D level was 25.9±7.8 and 29.1±8.6 (p value 0.139) in children with African (n=60) and mixed ancestry (n=76) respectively.

8.0 DISCUSSION

In this hospital-based unmatched case control study Vitamin D deficiency was present in 11(16.2%) of children with epilepsy compared to 6(8.8%) in the control group. Vitamin D insufficiency was present in 30(44.1%) in children with epilepsy compared to 27(39.7%) in the control group. Children with epilepsy on enzyme inducing AEDs had lower mean Vitamin D level, lower mean serum Phosphate and a higher mean Parathyroid hormone levels compared to the control group.

This study showed that Vitamin D deficiency or insufficiency is common in children with epilepsy on AEDs in the Western Cape, occurring in 60.3%. Similar results were found in other studies done in Korea, Australia and Denmark where the prevalence of vitamin D deficiency or insufficiency in children with epilepsy ranged between 50-66% (Fong and Riney, 2014; Lee et al., 2015; Vestergaard et al., 2004). This study further reiterates that vitamin D deficiency is common in children with epilepsy, thus where there is capacity all patients on AEDs should be investigated for Vitamin D deficiency and supplemented accordingly.

In this study children with epilepsy on AEDs had significantly lower levels of serum calcium and phosphate levels compared to the control group. Similar findings were seen in a case control study in Nigeria which found children with epilepsy had lower serum calcium levels compared to the control group but there was no difference in serum phosphate between the two groups (Oladipo et al., 2007). A study in Turkey also found children on carbamazepine and sodium valproate had lower serum calcium levels compared to the control group (Erbayat Altay et al., 2000). Other reports have also shown hypocalcaemia and hypophosphataemia are associated with the use of AEDs (Ali et al., 2004; Erbayat Altay et al., 2000; Pack et al., 2004; Sheth, 2004). AEDs particularly cytochrome P450 enzyme inducers cause reduction of Vitamin D leading to impaired bone metabolism thus causing low serum calcium and phosphate.

This study showed that children with epilepsy on cytochrome P450 enzyme inducing AEDs had higher mean parathyroid hormone levels compared to those on cytochrome P450 enzyme inhibitors. Children with epilepsy on enzyme inducing AEDs had also higher parathyroid hormone levels compared to the control group. Similar findings were shown in cross sectional studies in Malaysia and Australia were children with epilepsy on AEDs had elevated parathyroid hormone levels (Fong et al., 2016; Fong and Riney, 2014). In our study serum vitamin D levels were significantly lower in children on cytochrome P450 enzyme inducing AEDs compared to the control group. Serum Vitamin D were also lower in children with cytochrome P450 enzyme inducing AEDs compared to those on cytochrome P450 enzyme inhibitors, however the difference did not reach statistical significance. The AEDs, particularly cytochrome P450 enzyme inducers cause a reduction in the Vitamin D level leading to a secondary hyperparathyroidism as demonstrated in this study.

The mean Vitamin D_2($25(OH)D_2$) and mean Vitamin D_3($24,25(OH)_2D_3$) were significantly lower in children with epilepsy on enzyme inducing AEDs compared to those on enzyme inhibitors and the control group. Vitamin D_2 and Vitamin D_3 are thought to be metabolites of Vitamin D metabolism (Beckman et al., 1996). Enzyme inducing AEDs cause increased destruction of Vitamin D, thus leading to little formation of Vitamin D_2 and Vitamin D_3. This is the first study to report that children on enzyme inducing AEDs have lower levels of Vitamin D_2 and D_3 levels probably as result of increased destruction of Vitamin D.

This study did not show difference in the number of AEDs used between children with Vitamin D deficiency and those without. This is contrary to other studies which have shown polytherapy is associated with lower vitamin D levels (Bergqvist et al., 2007; El-Hajj Fuleihan et al., 2008; Fong et al., 2016; Guo et al., 2001; Nettekoven et al., 2008). This is probably because our cohort was recruited from a tertiary center which comprises of children with the severe forms of epilepsy and most of these children are on multiple AEDs.

The type of seizures the child experiences, either focal or generalized were not associated with the Vitamin D level in this study. Similar findings were reported in cross sectional studies of children with epilepsy conducted in Australia and Malaysia (Fong et al., 2016; Fong and Riney, 2014). The data from this study further highlights that the seizure type is not associated with the vitamin D level.

This study also showed that Vitamin D level was not associated with whether a child had a known epilepsy syndrome with a known aetiology or not. Similar results were demonstrated by a hospital based cross sectional study conducted in Australia (Fong and Riney, 2014). This further emphasizes that Vitamin D level is not affected by the type of epilepsy or its aetiology but rather by the choice of AEDs a patient receives.

Similar to the studies conducted in Australia and Malaysia, this study showed no difference in the dietary intake between children with epilepsy with Vitamin D deficiency and those without (Fong et al., 2016; Fong and Riney, 2014). The primary source of vitamin D is sunshine and dietary intake contributes less than 10% of the requirement (*Braunwald E, Harrison TR. Harrison's principles of internal medicine. 15th ed. New York: McGraw-Hill, Medical Pub. Division; 2001*, n.d.). This study further reiterates that the key lifestyle determinant of the vitamin D level is sun exposure and not dietary intake.

Studies from South East Asia have shown that ethnicity does affect the vitamin D levels. A hospital based cross sectional study in Malaysia showed children of Indian ethnicity had lower Vitamin D levels compared to the other ethnic groups (Fong et al., 2016). A multinational study conducted in Indonesia, Thailand, Vietnam and Malaysia also showed children of Indian ethnicity had lower Vitamin D levels compared to the other ethnic groups (Poh et al., 2016). In contrary this study showed no difference in the mean Vitamin D level between the children of

African and mixed ancestry. Thus ethnicity may not be a contributing factor to the Vitamin D level in children in the Western Cape.

This is the first study to assess Vitamin D levels in children with epilepsy in Western Cape. It is the first study to assess not only parameters of bone metabolism but also other Vitamin D metabolites and dietary intake. Previous studies have only assessed Vitamin D levels and other parameters of bone metabolism without including the vitamin D metabolites (Fong et al., 2016; Fong and Riney, 2014; Nettekoven et al., 2008).

9.0 STUDY LIMITATIONS

Children with Vitamin D deficiency did not undergo DEXA scan as part of the study due to financial constraints however they were referred to the attending clinicians for further management.

This was unmatched case control study, the control group was significantly younger and this may have affected the results. However, this may only have small effect on the results since age does not affect bone metabolism before puberty.

10.0 CONCLUSION

1. Vitamin D deficiency is common in children with Epilepsy on AEDs.
2. Children with epilepsy on enzyme inducing AEDs have lower mean of Vitamin D levels compared to those on enzyme inhibiting AEDs.

11.0 RECOMMENDATIONS

All children with Epilepsy on AEDs should be investigated for Vitamin D deficiency (using 25(OH) vitamin D) and managed accordingly.

Vitamin D supplementation should be offered to children with Vitamin D deficiency.

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13. AUTHOR CONTRIBUTIONS

Edward Kija, Jo Wilmshurst, George Van der Watt, Shihaam Cader and Steve Delport were involved in conceiving and designing of the study. Edward Kija and Shihaam Cader collated the data. Barry Gidal and Marc Drezner facilitated the analysis of Vitamin D levels. Edward Kija

and Alex Shapson-Coe conducted the data analysis. Edward Kija wrote the first draft and Jo Wilmshurst reviewed the draft.

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APPENDIX 1: QUESTIONNAIRE

A. PATIENT PARTICULARS

1. Date of enrolment……
2. Name……..
3. RXH No…….
4. Tel No…….
5. Type of Participant
    a. Cases
    b. Controls
6. For the controls, reason for surgery…….
7. Study Code……
8. Date of Birth…….
9. Age……..
10. Sex
    a. M
    b. F
11. Residence……..
12. Skin Colour
    a. Dark skin
    b. Pale skin

13. School……..
    a) Special School……..
    b) Normal school……
14. Grade…….
15. Average duration of sun exposure per day
    a. 0 to 30 minutes
    b. 30 to 60 minutes
    c. >60 minutes
    d. Not at all
    e. Other, specify……

B. HISTORY

16. Pregnancy
    a) Term
    b) Premature
17. Birth weight……..
18. Apgar scores……..

19. Development Quotient…….
20. Learning difficulty……
21. Behaviour
    a) Abnormal
    b) Normal
22. If Abnormal, specify……

23. Age at onset of seizures……(months)
24. Seizure semiology
    a. Focal
    b. Generalized
        i. Tonic
        ii. Clonic
        iii. Tonic – Clonic
        iv. Atonic
        v. Myoclonic
        vi. Absence
25. Epilepsy Syndrome
    a. Y
    b. N
    c. If Y, Name of the Syndrome……..

12. Development of other seizures

No	Type of seizure	Age of onset	Average Seizure free period(months)

26. Electroencephalogram Findings

No	Date	Age of the Patient (months)	Finding

27. **Current AED Medication(s)**

No	Name	Age at onset (months)	Dose(mg/Kg)

28. **Previous AED Medication**

No	Name	Age at onset (months)	Dose (mg/kg)

29. Stigmata of Abnormal bone metabolism

Sign/Symptom	Yes	No
Pain		
Splayed wrists		
Rachitic rosary		

30. Feeding Assessment:

No	Food Source	Frequency/Week	Frequency/Month
1.	Fish		
2.	Beef		
3.	Eggs		
4.	Cheese		
5.	Cod liver oil		
6.	Milk, Yoghurt		

C. INVESTIGATIONS

No	Investigation	Level
1.	Serum Calcium	
2.	Serum Phosphate	
3.	Vitamin D	
4.	Parathyroid hormone	
5.	Urinary DPD	
6.	Urinary creatinine	
7.	ALT	
8.	AST	

Study Code…… Date……

APPENDIX II: Consent form-Cases

Invitation to participate in the study to determine abnormalities of bone metabolism in children with Epilepsy at Red Cross War Memorial Children's Hospital, Cape Town South Africa.

My name is Dr. Edward Kija a Senior Registrar in Neurology at Red Cross War Memorial Children's Hospital. I am conducting a study to determine the abnormalities of bone energy turnover or "metabolism" in children with Epilepsy at Red Cross War Memorial Children's Hospital. I would like to ask for your permission that I approach your child to seek his/her permission to take part in this study. I will explain to you the study aims, benefits and risks and answer any questions you may have

Study Aims

The aim of this study is to understand the changes in bone metabolism in children with Epilepsy at Red Cross War Memorial Children's Hospital.

How to participate in the study

To be part of the study we will need to ask a few questions about your child's health and to record his weight and overall health as well as ask about his food intake. A blood sample will be taken to measure his or her levels of "bone metabolism" (this will be for his or her calcium, phosphate, vitamin D and parathyroid hormone). You will not pay for anything extra to participating in this study.

Confidentiality

All the information collected as part of this study will be kept strictly confidential, in other words only the doctors doing the study will be able to see it. Your child's details will not be made public and your involvement in the study will remain anonymous, so nobody else will know.

Benefits

If you (Parents/Guardians) agree to include your child in this study, your child could benefit from knowing if he or she has abnormalities in bone metabolism and this will allow treatment for the problem to be started. Problems in bone metabolism can lead to pain as well as the risk of bones breaking easily and healing badly.

Risks

The blood samples taken in this study are standard tests done in hospital. Your child may experience pain and infection at the site of the blood sample. However all care will be taken to ensure this does not occur.

Rights to withdrawal

Your involvement in this study is entirely your own choice. If you decide not to involve your child in this study you will not affect the care and treatment of your child in this hospital. You can withdrawal from this study at any time even after you have given consent.

Who to Contact

For further enquiries please contact the following
Dr. Edward Kija
Department of Neurology
Red Cross War Memorial Children's Hospital
Tel: 021 658 5434
Email: edwardkija@yahoo.co.uk

Prof. Jo Wilmshurst
Department of Neurology
Red Cross War Memorial Children's Hospital
Tel: 021 658 5434
Email: jo.wilmshurst@uct.ac.za

Chairman of the Human Research Ethics Committee
University of Cape Town
Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Tel: 021 406 6338
Email: Iamees.emjedi@uct.ac.za

I……..the parent/guardian of…….have read/been told and understood the contents of this form. All my questions have been answered. I agree/disagree my child to participate in this study.

…….. …….. …… ……..

Parent's/guardian signature Date Investigator's signature Date

Study Code…… Date…….

APPENDIX III: Consent form-Controls

Invitation to participate in a study on abnormalities of bone metabolism in children with Epilepsy at Red Cross War Memorial Children's Hospital, Cape Town South Africa.

My name is Dr. Edward Kija a Senior Registrar in Neurology at Red Cross War Memorial Children's Hospital. I am conducting a study to look at bone energy turnover or "metabolism" in children with Epilepsy at Red Cross War Memorial Children's Hospital. I would like to ask for your permission that I approach your child to seek his/her permission to take part in this study. I will explain to you the study aims, benefits and risks and answer any questions you may have

Study Aims

The aim of this study is to determine the abnormalities of bone metabolism in children with Epilepsy at Red Cross War Memorial Children's Hospital.

How to participate in the study

Your child is here for a routine surgical procedure. As part of this procedure a tube will need to be put into one of his or her blood vessels (veins) and blood will be taken for routine investigation. This would need to happen whether your child took part in this study or not. We would like to ask your permission that we seek your child's permission for an additional sample of his/her blood to be taken at the same time to be used to compare the results of bone metabolism to the children we are studying who have Epilepsy. Your child's blood results will be used as our normal "baseline" values for children who are from the Western Cape.

Confidentiality

All information collected in this study will be kept strictly confidential. Only the doctors doing the study will see the results. . Your child's personal particulars will not be made public and your participation will remain anonymous.

Benefits

There is no direct benefit to you (as parents/guardians) or your child but we hope that the results from this study will help us care for children with Epilepsy better. If your child's results do show problems with his or her bone metabolism we will immediately let your doctor know and will recommend how they should respond.

Risks

Your child already needs the line inserted into his or her vein (blood vessel) and the routine bloods to be taken. The additional 3 mls (half a tea-spoon) of blood will not put your child at any risk. No extra procedure is done to your child as part of this study. Your child may experience discomfort and infection at line insertion site. However all precautions will be taken to ensure this does not occur.

Rights to withdrawal

Your choice to be part of this study or to withdraw from it is entirely voluntary, you may make these decisions at any time, even after giving consent (permission for your child to take part in the study). If you decline to participate in this study you will not compromise the care and treatment of your child in this hospital.

Who to Contact

For further enquiries please contact the following

Dr. Edward Kija
Department of Neurology
Red Cross War Memorial Children's Hospital
Tel: 021 658 5434
Email: edwardkija@yahoo.co.uk

Prof. Jo Wilmshurst
Department of Neurology
Red Cross War Memorial Children's Hospital
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I……..the parent/guardian of…….have read/been told and understood the contents of this form. All my questions have been answered. I agree/disagree my child to participate in this study.

…….. …….. …… ……....

Parent's/guardian signature Date Investigator's signature Date

Study Code…… Date……

APPENDIX IV: Assent Form-Cases

Invitation to participate in the study to determine abnormalities of bone metabolism in children with Epilepsy at Red Cross War Memorial Children's Hospital, Cape Town South Africa.

My name is Dr. Edward Kija, a senior doctor at Red Cross War Memorial Children's Hospital. I am doing a study to determine how healthy the bones are in children with Epilepsy at Red Cross War Memorial Children's Hospital. I would like to ask for your permission to take part in this study. I will explain to you why we want to do this study, benefits, risks and I will answer any questions you may have.

Study Aims

The aim of this study is to determine how healthy the bones are in children with Epilepsy at Red Cross War Memorial Children's Hospital.

How to participate in the study

For you to take part in this study we will ask a few questions about your health, record your weight and ask about your food intake. A blood sample will be taken to measure the health of your bones.

Confidentiality

All the information collected as part of this study will be kept confidential, in other words only the doctors doing the study will be able to see it. Your details will not be made public and nobody else will know of your involvement in the study.

Benefits

If you agree to take part in this study, you could benefit from knowing if your bones are not healthy and this will allow treatment for the problem to be started. Unhealthy bones can lead to pain as well as the risk of bones breaking easily and healing badly.

Risks

The blood samples taken in this study are standard tests done in hospital. Your may experience pain and infection at the site of the blood sample. However all care will be taken to ensure this does not occur.

Rights to withdrawal

Your involvement in this study is entirely your choice. If you decide not to be part of the study you will not affect your care and treatment in this hospital. You can withdrawal from this study at any time even after you have given consent.

Who to Contact

For further enquiries please contact the following
Dr. Edward Kija
Department of Neurology
Red Cross War Memorial Children's Hospital
Tel: 021 658 5434
Email: edwardkija@yahoo.co.uk

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I……..the parent/guardian of…….have read/been told and understood the contents of this form. All my questions have been answered. I agree/disagree my child to participate in this study.

…….. …….. …… ……..

Parent's/guardian signature Date Investigator's signature Date

Study Code…… Date…….

APPENDIX V: Assent Forms -Controls

Invitation to participate in a study on abnormalities of bone metabolism in children with Epilepsy at Red Cross War Memorial Children's Hospital, Cape Town South Africa.

My name is Dr. Edward Kija, a senior doctor at Red Cross War Memorial Children's Hospital. I am doing a study to determine how healthy the bones are in children with Epilepsy at Red Cross War Memorial Children's Hospital. I would like to ask for your permission to take part in this study. I will explain to you why we want to do this study, benefits, risks and I will answer any questions you may have.

Study Aims

The aim of this study is to determine how healthy the bones are in children with Epilepsy at Red Cross War Memorial Children's Hospital.

How to participate in the study

You are here for a routine surgical procedure. As part of the in this procedure a small tube will need to be put into one your blood vessels (veins) and blood will be taken for routine investigation. This would need to happen whether you take part in this study or not. We would like to ask your permission an additional sample of your blood to be taken at the same time to be used to compare the results of bone metabolism to the children we are studying who have Epilepsy. Your blood results will be used as our normal "baseline" values for children who are from the Western Cape.

Confidentiality

All the information collected as part of this study will be kept confidential, in other words only the doctors doing the study will be able to see it. Your details will not be made public and nobody else will know of your involvement in the study.

Benefits

There is no direct benefit to you or your parents/guardians but we hope that the results from this study will help us care for children with Epilepsy better. If your do

show problems with bone metabolism we will immediately let your doctor know and will recommend how they should respond.

Risks

You already need the line inserted into your vein (blood vessel) and the routine bloods to be taken. The additional 3 mls (half a tea-spoon) of blood will not put you at any risk. No extra procedure is done to you as part of this study. You may experience discomfort and infection at line insertion site. However all precautions will be taken to ensure this does not occur.

Rights to withdrawal

Your choice to be part of this study or to withdraw from it is entirely voluntary, you may make these decisions at any time, even after giving consent (permission for you to take part in the study). If you decline to participate in this study you will not compromise your care and treatment in this hospital.

Who to Contact

For further enquiries please contact the following
Dr. Edward Kija
Department of Neurology
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Tel: 021 658 5434
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I…… ……have read/been told and understood the contents of this form. All my questions have been answered. I agree/disagree to participate in this study.

…….. …….. …… ……..

Parent's/guardian signature Date Investigator's signature Date