MMED DISSERTATION

BONE METABOLISM IN AMBULATORY, PREMENOPAUSAL WOMEN USING ANTIEPILEPTIC DRUGS, ATTENDING THE NEUROLOGY CLINIC AT THE KENYATTA NATIONAL HOSPITAL.

A dissertation submitted in part fulfilment for the degree of Master of Medicine in

Internal Medicine, University of Nairobi.

By

Dr Judith K Kwasa
2007

DECLARATION

I hereby declare that this is my original work and has not been presented for a degree in any other university.

Nwasa 22/6/07

This dissertation has been submitted for examination with our approval as supervisors.

DR T.O. KWASA
CONSULTANT NEUROLOGIST
SENIOR LECTURER, DEPARTMENT OF CLINICAL MEDICINE AND THERAPEUTICS
UNIVERSITY OF NAIROBI.

23/5/07

DR A. AMAYO
CONSULTANT PATHOLOGIST
SENIOR LECTURER, DEPARTMENT OF HUMAN PATHOLOGY
UNIVERSITY OF NAIROBI.

27.6.07

DR MUIA NDAVI
CONSULTANT OBSTETRICIAN GYNEACOLOGIST
SENIOR LECTURER, DEPARTMENT OF OB/GYN.
UNIVERSITY OF NAIROBI.

8.6.07

DEDICATION

I dedicate this dissertation to my late father Prof S. O. Kwasa. A man whose thirst for knowledge could not be quenched, and whose joy it was to see people he helped, eventually achieve more than himself. You will always be my source of inspiration.

ACKNOWLEDGEMENTS

I would like to offer my sincere gratitude to the following people, without whose input, this work would not be complete.

My supervisors, Dr T Kwasa, Dr A Amayo and Dr Muia Ndavi, for their input, guidance and patience.

Mr Fred Oyugi, for his technical support in demystifying the statistical aspects involved in this study.

Mrs Murimi, my indispensable research assistant.

Mr Maina, for his assistance with processing of the Lab work.

Mr Wachira, the Chief Radiographer at Nairobi Hospital, and the entire staff at the Nairobi Hospital Radiology Department, who ably carried out all the BMDs.

My soul mate, Chebii, for believing in me even when I did not believe in myself, and for his tireless help in giving me some form of focus in my nebulous endeavours.

My son, David,.. My pride and joy.

Last and definitely not least, My Mother Margaret, Brother Clem and Sister Carol; for being a constant source of positive thoughts, laughter and prayer.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

ABBREVIATIONS

AEDs	Anti Epileptic Drugs
KNH	Kenyatta National Hospital
CBZ	Carbamazepine
PHT	Phenytoin
VPA	Valproate
PB	Phenobarbital
PRM	Primidone
LTG	Lamotrigine
BMD	Bone Mineral Density
PTH	Parathyroid Hormone
DEXA	Dual Energy X-ray Absorptiometry
FDA	Food and Drug Administration
SD	Standard Deviation
CT	Computerized Tomography

ABSTRACT

BACKGROUND

Long term antiepileptic drug use causes multiple abnormalities in calcium and bone metabolism that have been described in both institutionalized and ambulatory patients, in the Western set-up.

On average at Kenyatta National Hospital, 30 to 35% of the total numbers of patients seen weekly at the Neurology clinic are women with epilepsy.

RATIONALE

Exposure to anti-epileptic drugs puts women at a higher risk for osteoporosis, before they reach menopause.

No studies have been published locally to describe the effect of long term AED use on women's bone health.

OBJECTIVE

The broad objective of this study was to assess bone metabolism in ambulatory females of reproductive age on antiepileptic drugs, at KNH.

METHODS

This was a cross-sectional comparative study that explored the relationship between Bone health and long-term treatment with anti epileptic drugs in women of reproductive age. Fifty seven women on drugs for more than 1 year were compared with 53 age-matched controls. Biochemical markers of bone metabolism were measured, namely serum Calcium, Phosphate, Alkaline Phosphate and Urinary Calcium Excretion. Bone Mineral Density

measurement at the Lumbar Spine was undertaken on a limited number of subjects due to financial constraints. (24 patients and 24 controls) Patients were, as closely as possible, matched for age, weight and height with an otherwise healthy control.

RESULTS

The mean duration of treatment for epilepsy was 8.8years (±6.3). Majority of the patients were on enzyme inducing drugs, either alone or in combination with non-enzyme inducers (98.2%). There was a significantly lower mean serum calcium and a higher alkaline phosphatase level among the patients (P=0.002 and 0.0001 respectively) than among the controls. The urinary marker of bone loss (mean Urine calcium excretion) was also significantly raised among the patients (P=0.003). The mean Lumbar BMD T-score results were not significantly different in the two groups.

CONCLUSION

Long-term antiepileptic drug use significantly affects biochemical parameters of bone metabolism. These effects on bone biochemistry markers were not reflected in Lumbar spine BMD

RECOMMENDATIONS

Further studies with larger sample sizes are required to fully elucidate the impact of AEDs on bone metabolism.

Health care workers should be aware of the possible side effects of AEDs on bone health; especially as regards women, who are prone to age-related osteoporosis.

1.0 INTRODUCTION AND LITERATURE REVIEW

Epilepsy is a common neurological condition affecting 1 in every 100 individuals, both children and adults. With the exception of epilepsy related to head trauma, the incidence is equal for men and women. The burden of disease is only made bigger by issues such as the need to take medication every day for years or even a lifetime, the concern that a seizure could occur at any time, and the social and economic hardships that accompany this misunderstood condition. (1)

1.01 DRUGS AND EPILEPSY

Although most people with epilepsy become seizure free with appropriate therapy, 30% to 40 % of patients will continue to have seizures despite the use of AEDs either alone or in combination(2). In the local setting, general practitioners play a vital role in the diagnosis and treatment of epilepsy. This is because, only a small percentage of patients with new-onset epilepsy are initially seen by a neurologist, and this translates to a vast majority of patients being on long term management under care of a general practitioner or general physician at best.

Prior to 1993, the choice of anticonvulsant medication was limited to "traditional" drugs: Phenobarbital, Phenytoin, Carbamazepine, Valproate and Primidone. Although these drugs have the advantage of familiarity as well as proven efficacy, many patients are left with refractory seizures as well as intolerable adverse effects. Since 1993, 8 new medications have been

approved by the US Food and Drug Administration (FDA), expanding treatment options. These are Felbamate, Gabapentin, Lamotrigine, Topiramate, Tiagabine, Levetiracetam, Oxcarbazepine and Zonisamide. The newer AEDs offer the potential advantages of fewer drug interactions, unique mechanisms of action, and a broader spectrum of activity. (3)

1.02 WOMEN AND EPILEPSY

Epilepsy is a common neurologic disorder affecting women during the reproductive years. Seizures and some AEDs can compromise reproductive health, carbohydrate and bone metabolism (4). As a general rule, the "traditional" drugs (see above) are associated with a worse side-effect profile than the newer AEDs. The side effect profile is related to the cytochrome P450 enzyme system. The AEDs most commonly associated with altered bone metabolism and decreased bone density are inducers of the cytochrome P450 enzyme system like Phenytoin, Carbamazepine, Primidone and Phenobarbital. (5,6,7)

Women with epilepsy face additional challenges. Some AEDs reduce levels of physiologic ovarian sex steroid hormones and may reduce the efficacy of contraceptive steroids. Women with epilepsy have a greater risk for syndromes associated with infertility, such as hypothalamic-pituitary axis disruption, polycystic ovary-like syndrome and anovulatory cycles.
Bone loss related to AEDs is more likely to lead to pathological fracture in postmenopausal women due to the dual insult of hormonal deficiency and AED effect on bone metabolism. (8)

In addition, women with epilepsy taking AEDs are at higher risk for pregnancy complications related to seizures, morphological abnormalities in offspring, and, perhaps, neurodevelopmental compromise. Unfortunately, most physicians are not knowledgeable about these health risks. (1, 4)

1.03 EFFECTS OF AEDs ON WOMENS' BONE HEALTH

Women genetically have a smaller bone mass and some AEDs may compromise bone health and alter bone mineral metabolism. Women using PHT, PB and perhaps CBZ and VPA are at higher risk for bone disorders such as osteopenia, osteomalacia and fractures. (5-7) Bogliun et al, in a prospective study evaluating the risk of hip fractures in women >65 years, found that women taking AEDs for more than 2 years were two times more likely to have a hip fracture. (1, 8) These results have not been reproduced in Premenopausal women.

Given available data, women with epilepsy should engage in good bone health practices, including adequate daily intake of calcium (1200mg/day) and vit D, gravity-resisting exercise, and bone density scans if they have taken PHT, CBZ or VPA for $\geq$ 5 years. Bone density scans should be repeated at 3- to 5-year intervals in Premenopausal women. (1)

1.04 EFFECT OF AEDs ON BONE METABOLISM IN EPILEPTIC PATIENTS

Long-term Antiepileptic Drug use causes multiple abnormalities in Calcium and Bone metabolism. From the first study in the 1960s, a growing body of

literature indicates an association between AEDs and bone disease, including histologic, radiographic, and biochemical evidence. Early reports revealed bone disease as evidenced by pathological biopsies which revealed decreased bone trabeculation, but these were predominantly studies carried out on institutionalized patients. (9,10) In institutionalized patients, there exists many confounding variables e.g. inadequate sunlight exposure, poor diet and limited exercise. These are likely to adversely affect bone metabolism and hence influence the findings.

Recent studies in ambulatory patients describe biochemical and radiographic abnormalities consistent with decreased bone mineral density (BMD) and disorders of bone mineral metabolism (5-7, 11-16). Sato et al found a 13% reduction in Bone Mineral Density in 40 ambulatory adult patients on PHT for more than a year, as compared to controls(13). Farhat et al found that 59% of their 42 adults on AEDs for at least 6 months had osteopenia at either the spine or hip. (14)

1.05 POSSIBLE MECHANISMS OF AED-ASSOCIATED BONE DISEASE

Several theories have been proposed to explain the link between AEDs and bone disease. Hepatic induction of the cytochrome P450 enzyme system leading to increased catabolism of Vitamin D is the principle mechanism reported. (5, 17-18) However, it does not explain the findings described in patients receiving other medications, such as VPA, an inhibitor of cytochrome P450 enzyme system. In addition, the finding of vitamin D

deficiency has not been demonstrated in all studies, and evidence of bone turnover is found independent of vitamin D deficiency. (5, 19-20)

Other possible mechanisms include

- Direct effect on bone cells, including impaired absorption of calcium, and inhibition of response to PTH
- Hyperparathyroidism
- Calcitonin Deficiency (17)

AEDs that induce hepatic cytochrome P450 enzymes may cause increased conversion of vitamin D to polar inactive metabolites in the liver microsomes, reducing bioavailable vitamin D. Decreased biologically active vitamin D leads to decreased absorption of calcium in the gut, resulting in hypocalcaemia and an increase in circulating parathyroid hormone (PTH). PTH then increases the mobilization of bone calcium stores and subsequent bone turnover

AEDs may interfere with intestinal absorption of calcium. This effect would most likely be indirect, through vitamin D, but a direct effect has been postulated. Impaired absorption would lead to hypocalcaemia and feedback hypersecretion of PTH. Koch et al in 1972, found markedly decreased calcium absorption in rats treated with Phenytoin but not with Phenobarbitone. (21) These results suggest that in patients treated with Phenytoin, impaired calcium absorption may play a role.

Evidence also exists for inhibition of the cellular response to PTH. Fetal rats treated with Phenytoin or Phenobarbitone demonstrated an impaired response to PTH. Inhibition of the bone resorptive response to PTH could lead to hypocalcaemia, a frequent finding in patients taking AEDs (21).

Hyperparathyroidism has also been suggested as a possible mechanism. This is most likely secondary hyperparathyroidism resulting from hypocalcaemia and hypovitaminosis D. However in one study; both male patients with normal vitamin D status (5) and subjects who were vitamin D depleted had evidence of hyperparathyroidism (19). Hyperparathyroidism can primarily activate bone resorption and, through a coupling phenomenon, secondarily activate bone formation.

A final postulated mechanism is calcitonin deficiency. Calcitonin is a hormone produced by the thyroid gland that inhibits osteoclast-mediated bone resorption. Calcitonin deficiency may therefore accelerate bone turnover. This deficiency has been demonstrated in a paediatric population (23-24).

1.06 BIOCHEMICAL CHANGES

Multiple biochemical abnormalities of bone metabolism are present in patients taking AEDs. (18) These can be summarised as follows;

Markers of bone formation have been assessed in patients receiving AEDs, which include alkaline phosphatase, osteocalcin, and the C-terminal extension peptide of type I procollagen. Alkaline phosphatase is the most commonly used marker of bone formation, and increases have been seen in both adults and children receiving AEDs. (5, 29, 30) Because serum total alkaline phosphatase is derived from bone, liver, and other sources, it lacks specificity in evaluating bone disease.

High levels of osteocalcin have been described. As a biological marker of bone formation, an elevation suggests increased bone turn over. (5, 20)

Significant elevations in the C-terminal extension peptide of type I procollagen has been seen in patients taking AEDs. (5, 20)

Specific markers of bone resorption can be measured in the urine and serum. Cross-linked carboxy-terminal telopeptide of human type I collagen is a serum marker of bone degradation, and hydroxyproline is a marker in the urine. These markers are elevated in patients with epilepsy receiving long-term AEDs (5) and after recent initiation of therapy. (20)

1.07 RADIOGRAPHIC CHANGES

Numerous techniques have been used to detect histologic and radiographic evidence of bone abnormalities in patients receiving AEDs, ranging from bone biopsies to the present gold standard in detection of decreased BMD, Dual Energy X-ray Absorptiometry. (DEXA) DEXA assesses predominantly trabecular bone, such as the spine, hip and the ribs, and measures the total mineral content. It is the most sensitive technique

available for assessing BMD, detecting a 5% decrement or less of bone mass. Quantitative CT BMD has a similar ability to predict fractures but requires a larger dose of radiation.

In the DEXA technique, two x-ray energies are used to estimate the area of mineralized tissue, and the mineral content is divided by the area, which partially corrects for body size. Since this correction is only partial, smaller people tend to have a lower-than-average BMD. Consequently it has become standard practice to relate the results to 'normal' values using T-scores (young population matched for age and gender) and Z-score (age adjusted T score) According to the World Health Organization, a BMD T-score below -2.5 SD is diagnostic of osteoporosis, whereas a T-score between -1 and -2.5 SD is diagnostic of osteopenia.

Several studies have measured BMD in adult patients receiving AEDs by using DEXA, finding significantly reduced BMD at the Ribs and Spine (7, 14), femoral neck (6,14, 15), and total hip (14) A prospective study quantified on-going bone loss in young men receiving AEDs, with the highest rate of bone loss in the young male skeleton. (15)

Females with epilepsy had significantly lower BMD at the femoral neck compared with other sites in one study. (11) The hip is the preferred site in most individuals, since it predicts the risk of hip fracture, the most important consequence of osteoporosis. In younger individuals however, such as peri menopausal women, spine measurements may be the most sensitive indicator of bone loss.

1.08 LOCAL STUDIES ON BONE HEALTH IN WOMEN

Odawa et al assessed 220 women to determine whether osteoporosis afflicts Black Kenyan women in their Peri menopausal and post menopausal periods. Only 43.7% of the post-menopausal women had normal BMD as compared to 78.6% of the pre-menopausal women who had a normal BMD. ($p<0.003$) (32)

Odula et al did a comparative study to compare the pattern of osteoporosis and osteopenia in three different racial groups of women. The results showed higher measures of BMD amongst the African woman, followed by the Asian woman and lowest BMD amongst Caucasians.(31) The significant difference in findings indicate that the BMD in the African woman seemed to be better despite the presence of known influencing factors e.g. dietary habits and parity, which would have a negative impact. Physical activity and the limited use of alcohol or tobacco among Africans, are factors that would favour higher BMD in the African women.

2.0 RATIONALE AND JUSTIFICATION

Epilepsy is a common neurologic disorder affecting women in the reproductive years and it is known that long-term antiepileptic drug therapy use causes multiple abnormalities in bone metabolism. As it is inevitable that these patients will be on long-term maintenance therapy, it can well be expected that they will be exposed to this documented side effect. Early exposure in these women, as they approach menopause, puts them more at risk of osteoporosis and potential bone fractures.

The fact that enzyme inducing drugs have a more serious effect on bone metabolism, than do non-enzyme inducers, suggests that some drug combinations need to be more closely monitored for this side effect, and intervention initiated early.

No studies had been done locally, and indeed, no studies had been published from Africa, to describe the effect of long-term AED use on women's bone health. Bone metabolism is affected by a variety of dietary and lifestyle habits, and the impact of long-term AED therapy on African women's bone health, may prove to be different considering the differences in BMD elicited in African women compared to Caucasian women. (31)
As there is already documented osteopenia of normally menstruating, premenopausal women in our set-up, long-term AEDs could significantly worsen this prevalence.

Knowledge of the magnitude of the problem would impact on the holistic approach to management of an epileptic patient on long-term AED use. The onset of future bone complications can be postponed, or even avoided, by early intervention. Intervention techniques may include calcium and vitamin D supplementation, biphosphonates, calcitonin, hormone replacement therapy, gravity resisting exercise and periodic bone density monitoring. It was against this background that this study was conducted.

3.0 OBJECTIVES

The broad objective of this study was to assess Bone Metabolism in Ambulatory Females of Reproductive age on Antiepileptic Drugs, at KNH.

The Specific Objectives were:

- To determine BMD in study subjects and controls using DEXA
- To measure blood markers of bone biochemistry in study subjects and controls. (Calcium, Phosphate, Albumin, and Alkaline Phosphate)
- To measure markers of bone turnover in urine of study subjects and controls. (Calcium- Creatinine ratio, urinary calcium excretion)
- To compare the biochemical and radiological findings in study subjects and controls.

HYPOTHESIS

BMD among women of reproductive age on AEDs was different compared to control subjects

RESEARCH QUESTIONS

I. Did women on AEDs have higher bone formation and bone turnover indices than control subjects.
   a. Biochemical indices of bone formation (ALP)
   b. Biochemical indices of bone turnover (Urinary excretion of calcium, Urine calcium creatinine ratio)

4.0 STUDY DESIGN AND METHODOLOGY

4.01 STUDY DESIGN

The study was a cross-sectional comparative study in which women of reproductive age on AEDs were compared to women in reproductive age who had not used AEDs.

4.02 STUDY AREA

The study was conducted at Kenyatta National Hospital, Neurology clinic.

4.03 STUDY POPULATION

This comprised women of reproductive age who had been on antiepileptic drugs for at least 1 year, and who were attending the neurology clinic. The control group consisted of patient escorts and friends. They were all women of reproductive age.

4.04 INCLUSION CRITERIA (PATIENTS)

- Epileptic women between the ages of 15 to 49 on antiepileptic drugs for at least 1 year
- Patients known to be normally active and ambulant
- Written and informed consent

4.05 INCLUSION CRITERIA (CONTROLS)

- Healthy, ambulant, normally menstruating females between the ages of 15 to 49 years.
- No previous use of AEDs
- Written and informed consent

4.06 EXCLUSION CRITERIA (PATIENTS AND CONTROLS)

- Patients known to have had a history of severe Renal, Liver, Cardiac or Thyroid/Parathyroid disease, Diabetes or malignancy.
- Current pregnancy and/or amenorrhea of 6 weeks
- Patients with prior history of bone fractures in the preceding 1 year
- Patients who had been bedridden in the previous 1 month for a period equal to, or exceeding 2 weeks.
- Patients with 2 week history of diarrhoea and/or malabsorption
- Patients known to be or have been on drugs affecting bone metabolism: Glucocorticoids, Calcium and Vit D supplements, Thiazide diuretics, Hormonal contraception, Biphosphonates or Calcitonin.

4.07 SAMPLE SIZE

The minimum sample size was 53 patients as calculated by the formula

$$n = 2\frac{(Z_\alpha + Z_\beta)^2 \times P \times (1 - P)}{(P_0 - P_1)^2}$$

Where

n = Sample size

$P = (P_0 + P_1)/2$

Z_α = Standard error from mean corresponding to 95% C.I. (1.96)

Z_β = Power of the test (90% = 1.282)

P_1 = Prevalence of osteopenia in the control population of 20% (0.2)

P_0 = Estimated osteopenia prevalence of 50% in study population (0.5)

There were 57 patients and 53 controls subjected to biochemical evaluation. Due to cost constraints, a smaller number of study subjects (24 patients and 24 controls) were subjected to radiological evaluation. This number was downsized due to cost implications. This number was obtained by reducing the power of the test to 80% Z_β and using a higher prevalence figure. (49)

Z_β = Power of the test (80% = 0.84)

P_0 = Estimated osteopenia prevalence of 60% in study population (0.6)

4.08 SAMPLING METHOD

Each week, the principal investigator and a research assistant visited the KNH Neurology clinic, and selected female patients on follow up for epilepsy, using a systematic sampling method as outlined in the patient recruitment section. If consent was obtained for the study, they were considered recruited. Three to five patients were recruited per week. This systematic selection continued each week until the desired sample size was achieved.

Controls were selected from patient escorts (neurology and gynaecology clinics) and friends, who fit the inclusion criteria and did not fall in the exclusion criteria, as outlined in the patient recruitment section.

On average, at the KNH, 30 to 35% of the total number of patients seen weekly at the neurology clinic, (~60 patients) are women with epilepsy. One week before data collection began, the research assistant was trained on how to fill the questionnaire and take relevant samples.

4.09 CLINICAL METHODS:

4.091 SUBJECT RECRUITMENT

4.0911 PATIENTS

Every female patient attending the clinic was evaluated for suitability. Each week, a list of all female patients booked for the clinic, with a diagnosis of epilepsy and in the identified age group was drawn up. From the list,

systematic sampling of every third subject was used to select 3 to 5 patients on each clinic day. These patients had the study explained to them and if they gave consent, were subjected to the screening questionnaire. (See appendix III) If no consent was given, the systematic sampling was repeated to select further patients for the study.

If the results of the screening questionnaire were acceptable, the patient was asked to sign a consent form and/or assent form, and if done, was considered recruited. It is at this stage that she was subjected to the study proforma. (See appendix) This selection was repeated each clinic day until the desired sample size was achieved.

4.0912 CONTROLS

Controls were also evaluated weekly. Each week, a similar number of controls was selected to match the patient numbers already picked. The controls were matched for age to the patients. Once a control subject was identified, she had the study explained to her and if she consented, was subjected to the same screening questionnaire as the patients. (See appendix III) If no consent was given, another control was identified after systematic sampling, and the procedure repeated.

If the results of the screening questionnaire were acceptable, the control was asked to sign a consent form and/or assent form, and if done, was considered recruited. It is at this stage that she was subjected to the study proforma. (See appendix III)

Random selection was used to select the 24 out of 53 patients/controls who eventually went for radiographic studies, using the priori approach. Study subjects and controls were numbered 1-57. A lottery method was used to select the 24 patients and controls who went for radiographic evaluation.

4.092 CLINICAL EVALUATION

History was taken and Physical examination performed on all the recruited patients as outlined in the study proforma (See appendix III)

This was aimed at:

1. Determining demographic data.
2. Establishing the current and previous intake of antiepileptic medications in patients on AEDs.

Controls were also subject to the history and physical examination procedure.

4.093 BIOCHEMICAL EVALUATION

Blood sample collection was carried out for determination of biochemical parameters on each subject, i.e. patients and controls. Same day samples were drawn from each subject, the technique of which is elaborated in the Laboratory Methods section. The parameters were analysed at KNH Renal laboratory using the Olympus AU 400 auto-analyser. Specific parameters assessed were:

- Serum calcium
- Serum phosphate
- Serum albumin
- Serum creatinine

Markers of bone resorption :

- Urine calcium Creatinine ratio
- Urinary excretion of calcium

Markers of bone formation:

- Serum Alkaline phosphatase

4.094 RADIOGRAPHY

Twenty four patients and 24 controls underwent CT Bone Mineral Density (BMD) assessment at the lumbar vertebrae (L2-L4). Bone mineral density was measured and *T* score (the difference in standard deviations between a given bone density value and peak bone density in the normal reference population) calculated. These studies were carried out at the Nairobi Hospital radiology department.

According to the World Health Organization, a BMD T-score below -2.5 SD is diagnostic of osteoporosis, whereas a T-score between -1 and -2.5 SD is diagnostic of osteopenia.

4.095 LABORATORY METHODS

Blood

6mls of venous blood was drawn into a plain (red-tube) bottle. The sample was allowed to clot, and was centrifuged at 1500g (3000rpm) for 15 minutes. 1ml of serum was transferred to 4 serum vials and stored at -20°C until further analysis.

Serum calcium, Creatinine, phosphate, albumin and alkaline phosphatase was measured using commercial reagents, on the Olympus AU 400 Auto analyser at the Department of Laboratory Medicine, Kenyatta National Hospital.

Urine

5mls of random mid-stream urine was collected in a universal bottle containing 2mls of conc. HCl. The sample was stored at 4°C until further analysis.

Urine calcium and Creatinine was measured on the Olympus AU 400. Urine calcium excretion was calculated using the formula outlined below

Calculations

Calcium was corrected for the albumin using the formula:

Corrected calcium(mmol/L)= Total calcium(mmol/L)+ 0.02[40-albumin(g/L)]

Urinary calcium excretion was calculated using the formula:

$$\text{Urinary calcium} = \frac{\text{Urine calcium(mmol/L)} \times \text{Serum creat(umol/L)}}{\text{Urinary creat(umol/L)}}$$

(Reference limit < 0.04mmol/L)

Quality Assurance

An aseptic technique was used for specimen collection to minimise pre-analytical errors. Procedures for specimen handling and storage were also adhered to. All equipment was calibrated according to manufacturers' specifications. Commercial control material was used to validate the calibrations. These were included in all the analytical runs. Results were accepted if the control values were within the expected ranges.

4.096 DATA ANALYSIS

Statistical analysis of data was undertaken using the Statistical Package for the Social Sciences (SPSS) version 11.5. Data was presented in the form of tables, graphs and pie charts.

Descriptive statistics such as means, medians and standard deviation were determined where applicable.

Comparison of results using Odds Ratio with a 95% Confidence Interval was done.

To assess the significance of differences in continuous data, the t-test was used. P values of <0.05 was considered significant.

5.0 ETHICS AND CONFIDENTIALITY

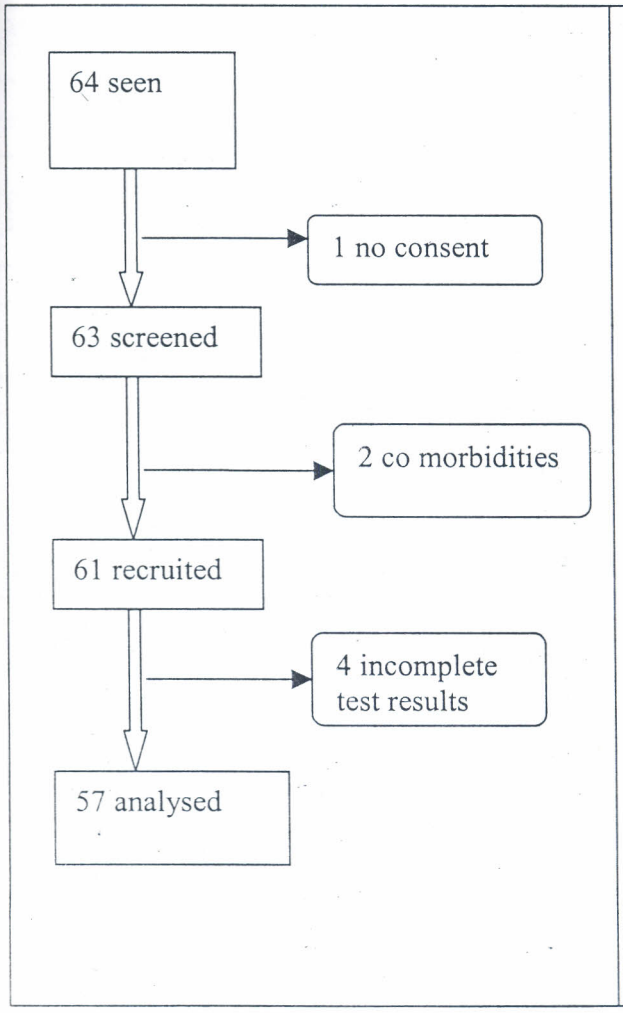
Ethical approval to carry out this study was obtained from the Kenyatta National Hospital Scientific and Ethical Review Committee and patients were enrolled for the study only after giving informed written consent. All information obtained from the study has been handled in confidence and used only for the intended purpose.

All laboratory and radiological outcomes were communicated to the primary doctor and the subject for relevant action.

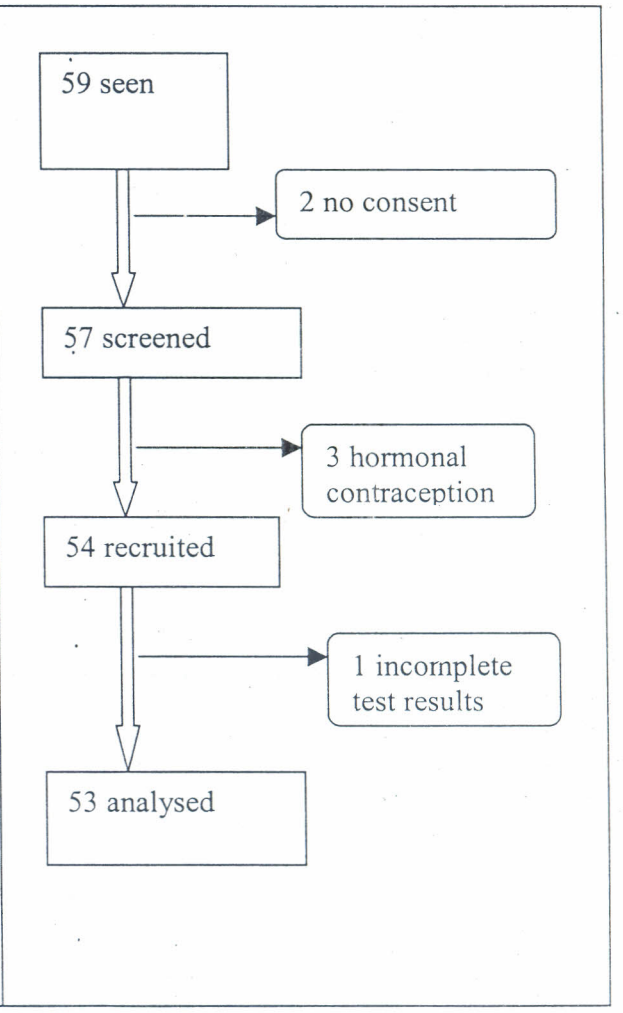
6.0 RESULTS

A total of 123 subjects were screened between February and October 2006. 13 were excluded for various reasons: 3 did not give consent, 2 had co morbid conditions, 3 were using hormonal contraception and 5 failed to give specimens for laboratory testing. A total of 110 subjects (57 patients and 53 controls) were recruited to the study as shown below. Serum and urine biochemical analyses were done for all study subjects, while 48 subjects (24 patients and 24 controls) had Bone Mineral Density (BMD) evaluation.

Patients' Flowchart



Controls Flowchart



6.01 DEMOGRAPHIC CHARACTERISTICS AND BMI

The results of the demographic characteristics and BMI for the two study groups are summarized in the table below.

Table 1: Demographic characteristics and BMI of study subjects

	Patients n=57	Control n=53	P Value
Age in years			
Mean (± SD)	26.4±7.7	28.7± 9.3	NS
Median (range)	26(15-45)	25(15-49)	
Age at Menarche (years)			
Mean (± SD)	13.96 ± 1.9	14.26 ± 4.4	NS
Median (Range)	14.8(10-16)	15.3(13-17)	
Marital status			
Single (%)	78.6	72.5	NS
Married (%)	19.6	23.5	NS
Separated (%)	1.8	3.9	NS
Peak Education Level			
None (%)	1.8	0	NS
Primary (%)	44.6	10.6	<0.001
Secondary (%)	46.6	34.0	0.001
Tertiary (%)	7.1	53.2	<0.001
Employment			
Employed (%)	24.5	48.9	0.036
Unemployed (%)	55.1	37.8	0.03
Never had formal employment (%)	18.4	13.3	NS
Retired (%)	2.0	0	NS
BMI (kg/m2)			
Mean (± SD)	23.4±3.3	24.6±3.6	NS
Median (Range)	22.6(16-30)	24.8(17-32)	

6.011 Age

The patients' age ranged from 15 years to 45 years with a median of 26 years. The mean age for patients was 26.4(±7.6) years. The mean age for controls was 28.7(±9.3) years with a median of 25 years (Table 1). There was no significant difference in the ages of patients and controls (p=0.164).

6.012 Age at Menarche

The patients had a mean age at menarche of 13.96(±1.9) yrs although the controls were slightly older at 14.26(±4.4), and, as shown in Table 1,the difference was not statistically significant. (p=0.062)

6.013 Marital Status

Most of the study subjects (78.6% and 71.1%of patients and controls respectively) were single. Among the patients, 19.6% were married and 1.8% were separated. Of the controls, 26.7% were married and 2.2% were separated. These differences were not significant. (Table 1)

6.014 Peak Education level

Most of the patients (63.5%) had achieved secondary education or higher. There was however one patient who had not received any formal education. Among the controls, 89.4% had received secondary education or above with majority (53.2%) having gone up to a tertiary level of education. There was no significant difference in education level in the two groups as shown in table 1.

6.015 Employment

As shown in Table 1, majority of patients (75.5%) were unemployed compared to 51.1% of controls who were unemployed. These differences were not statistically significant.

6.016 Body Mass Index (BMI)

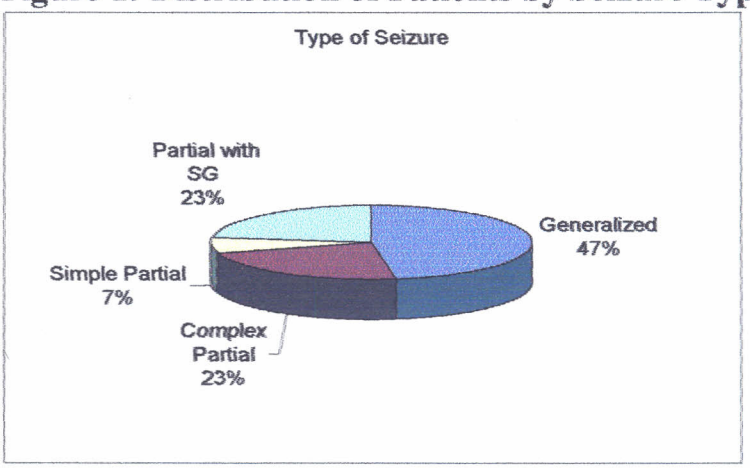
Table 1 shows the mean BMI for the patients as being 23.4(±3.3) kg/M^2. The mean BMI for the controls was 24.6(±3.6) kg/M^2 . The difference was not significant. (p=0.092)

6.02 DISTRIBUTION OF PATTERNS OF EPILEPSY AND TREATMENT

6.021 Seizure Type

Majority of patients (52.6%) had partial seizures as shown in Figure 1. These were mainly Complex Partial 22.8% and Partial with secondary Generalization 22.8%. Generalized tonic clonic seizures were seen in 47% of the patients.

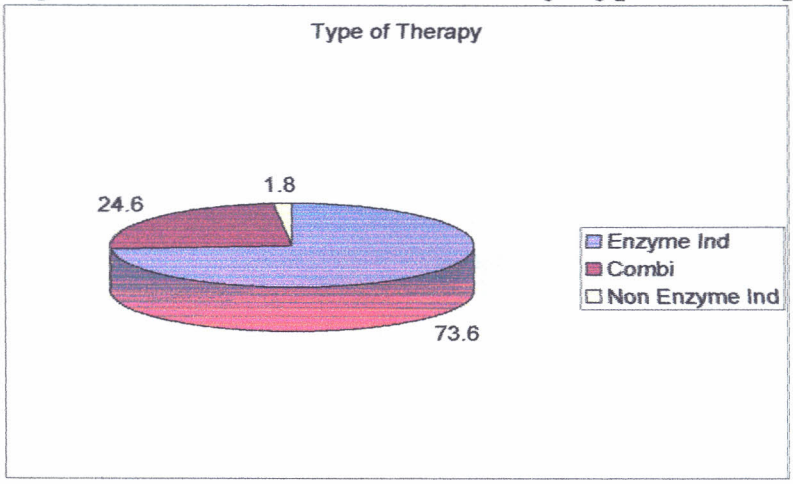
Figure 1: Distribution of Patients by Seizure Type (n=57)



6.022 Type and mode of Therapy

Majority of patients (75.4%) were on a single drug regimen. Most (73.6%) were using drugs broadly classified as Enzyme Inducers (Carbamazepine, Phenytoin or Phenobarbitone) while about a quarter of the patients were on a combination of both enzyme inducers and non-enzyme inducers (including Valproate and Clonazepam). 1 patient was strictly on a non-enzyme inducer, namely Gabapentin. (Fig 2)

Figure 2: Distribution of Patients by Type of therapy (n=57)

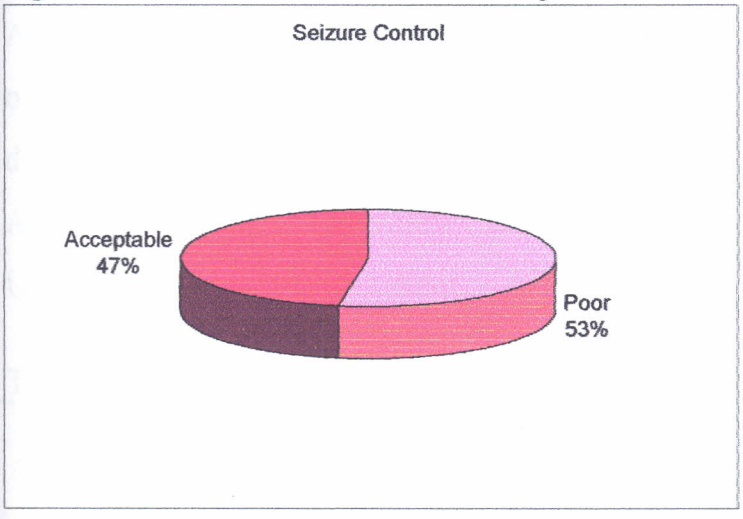


6.023 Seizure Control

This was roughly qualified by the number of seizures reported over the previous 6 month period: <2 seizures in 6 months was categorized as Acceptable control, while > or = 2 was categorized as Poor control. The patient's response to this question was compared with the documentation in the file and these results were found to be comparable.

As reported by patient, 52.6% had poor control, 47.4% had acceptable control. This was verified by file records (Figure 3)

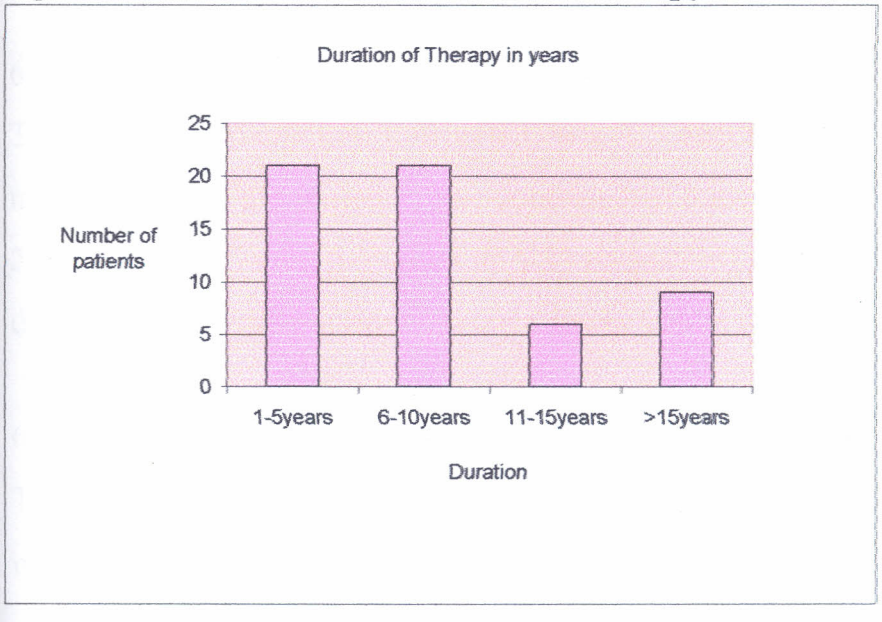
Figure 3: Distribution of Patients by Seizure Control (n=55)



6.024 Duration of Therapy

The mean duration of therapy was 8.8(±6.3) years with a median of 7 years (range 1-25). Majority of the patients (73.6%) had taken between 1 and 10 years treatment as shown in figure 4.

Figure 4: Duration of anticonvulsant Therapy in Years



6.03 LABORATORY PARAMETERS IN PATIENTS AND CONTROLS

All recruited patients and controls had serum and urine tests done, the results of which are summarized in Table 2 below. There was a significant difference between patients and controls, in levels of serum Calcium, Alkaline Phosphatase, Urinary Calcium, Excretion of Urinary Calcium and Urine Calcium Creatinine Ratio.

Table 2: Biochemical parameters in serum and urine of Patients and Controls

Laboratory characteristics	Patients n=57 (mean ± SD)	Controls n=51 (mean ±SD)	P Value
Calcium (mmol/L)	2.2±0.2	2.3±0.3	0.002
Calcium corrected (mmol/L)	2.2±0.2	2.4±0.3	0.002
Phosphate (mmol/L)	1.4±0.2	1.3±0.3	NS
Alk Phosphatase (u/L)	110.1±46.7	76.4±33.9	0.0001
Urine calcium (mmol/L)	1.7± 0.8	1.1± 0.6	0.005
Urinary calcium Excretion	0.04±0.03	0.02±0.02	0.008
Urine Ca/creatinine Ratio	0.38±0.27	0.24±0.21	0.005

6.031 Serum Calcium

The mean serum calcium (corrected) was 2.2 mmol/L± 0.2 with a median of 2.3 mmol/L among the patients. The controls had a mean serum calcium (corrected) of 2.4±0.3 mmol/L with a median of 2.33 mmol/L (Table 2). There was a significant difference with the patients having a lower value. (p=0.001)

6.032 Serum Phosphate

Table 2 shows that the patients had a higher mean serum phosphate of 1.41±0.2 mmol/L with a median of 1.44 mmol/L. For the controls, the mean serum

phosphate was 1.30±0.3 mmol/L with a median of 1.2 mmol/L. This difference was however not significant (p=0.055).

6.033 Serum Alkaline Phosphatase

Among patients, the mean serum alkaline phosphate was 110.1±46.7 mmol/L For the controls the mean was 76.4±33.9 mmol/L. (Table 2) The patients had a significantly higher value (p=0.0001), than the controls

6.034 Urine Calcium

Patients had a mean urine calcium of 1.7±0.8 mmol/L as depicted in Table 2, while controls had a mean urine calcium of 1.1±0.6 mmol/L. There was a significant difference with the patients having a higher value. (p=0.002)

6.035 Urinary Calcium Excretion

Patients had a mean urinary calcium excretion of 0.04 ±0.03 mmol/L while controls had a mean urine calcium excretion of 0.02 ±0.02 mmol/L. There was a significant difference with the patients having a higher value (p=0.008) as shown in Table 2.

About 30% patients and 12% controls had values >0.04 and this difference was not significant (p=0.104). An absolute value of >0.04 implies an increase in osteoclastic bone resorption.

6.036 Urine Calcium Creatinine Ratio

Table 2 shows that the mean urine calcium creatinine ratio was 0.38±0.27 with a median of 0.32 among the patients. For controls, the mean was 0.24±0.21 with a median of 0.21, where the normal range is 0.27 to 0.61. The patients therefore had a significantly higher value (p=0.005) as shown in Table 2.

About 17% patients and 9% controls had an absolute value outside the cut off of 0.61. This difference in numbers was however not significant. (p=0.308)

Table 3: Comparison of abnormal biochemical parameters between patients and controls

Parameter		Cases No.	Cntrls No.	OR 95% CI	P Value
Serum Corrected Calcium <2.1mmol/L	Abn	14	3	OR 4.75 (95%CI 1.28-17.80)	0.013
	Nor	43	44		
Serum Phosphate ≥1.4mmol/L	Abn	32	17	OR 2.56 (95%CI 1.17-5.60)	0.017
	Nor	25	34		
Serum Alk Phosphatase >120mmol/L	Abn	10	5	OR 1.96 (95%CI 0.62-6.17)	0.246
	Nor	47	46		
Urine Calcium Excretion >0.04mmol/L	Abn	13	5	OR 2.48 (95%CI 0.81-7.57)	0.104
	Nor	43	41		
Urine Ca/Creat Ratio >0.61	Abn	8	4	OR 1.92 (95%CI 0.54-6.80)	0.308
	Nor	48	46		

Table 3 shows that women using anti-epileptic drugs were 4.75 times as likely to have hypocalcaemia and 2.5 times as likely to have hyperphosphataemia, compared to non-epileptic women. These were statistically significant differences. The rest of the differences did not achieve statistical significance.

6.04 PATIENT SUBGROUP COMPARISONS

Table 4: Biochemical patterns by mode of therapy in study patients

Variable (mean)	Single therapy N=43	Multiple therapy N=14	P value
Corrected calcium	2.19±0.2	2.27±0.18	0.225
Alk Phosphatase	114.42±52.6	96.79±13.4	0.22
Serum Phosphate	1.41±0.19	1.42±0.2	0.781
Urine Calcium Excretion	0.04±0.03	0.03±0.03	0.82
Urine Ca Creat Ratio	0.37±0.24	0.40±0.35	0.7

As shown in Table 4, patients on single drug therapy had lower serum calcium, phosphate and urine calcium creatinine ratio. They had higher alkaline phosphatase and urinary excretion of calcium than those on multiple drugs. These differences, however, did not achieve statistical significance

Table 5: Biochemical parameters and type of seizure in study patients

Variable (mean)	Generalized Seizures N=27	Partial seizures N=30	P value
Corrected calcium	2.22±0.16	2.21±0.23	0.85
Alk Phosphatase	111.93±39.04	108.43±53.33	0.781
Serum Phosphate	1.46±0.20	1.36±0.18	0.054
Urine Calcium Excretion	0.04±0.03	0.04±0.03	0.99
Urine Ca Creat Ratio	0.38±0.27	0.38±0.27	0.95

On subgroup analysis, those having partial seizures had lower levels of serum calcium, phosphate and alkaline phosphatase, than those with generalized seizures, although these differences were not significant. There was no difference in urinary excretion of calcium, nor the urine calcium creatinine ratio (Table 5).

6.05 RADIOLOGICAL CHARACTERISTICS OF PATIENTS AND CONTROLS

Table 6: Lumbar Spine BMD T-Score in patients and controls

	Patients n=24	**Controls n=24**	**P value**
T-score			
Mean (±SD)	1.8±0.9	1.4±1.3	0.24
Median (Range -1.16 to 4.19)	1.6	1.1	

Forty eight individuals (24 patients and 24 controls) were randomly chosen to have Lumbar spine BMD study done.

The mean BMD T-score for the study group was 1.62 with a median of 1.51 (SD 1.03). The highest score was 4.19 and the lowest was – 1.16. Only one subject (who was a control) was found to have osteopenia. No subject had osteoporosis.

As shown in table 6, the mean BMD T-Score for the patients was 1.8±0.9 with a median of 1.59. This signified a normal BMD. Twenty four controls were analyzed and had a mean BMD T-score of 1.4±1.3 with a median of 1.1. This is within normal limits. There was no significant difference between the patients and controls. (P value= 0.24)

7.0 DISCUSSION

Osteoporosis is a common condition with major public health and economic implications (33). A number of risk factors have been proposed like age, female sex, estrogen deficiency, low calcium intake and also, exposure to anticonvulsant medication (34). In this study, women of reproductive age on AEDs showed a significant reduction in biochemical indices of bone mineralization, specifically serum calcium, compared to controls. The patients also showed an increase in biochemical markers of bone turnover, specifically serum alkaline phosphatase, urinary calcium-creatinine ratio and urinary excretion of calcium, over the controls. This difference was not duplicated in the radiological marker of bone health, Bone Mineral Density.

The patient and control groups were comparable in terms of age, BMI and age at menarche. These three characteristics are known to influence bone health in women. Osteoporosis occurs more frequently with increasing age as bone tissue is progressively lost. Low body weight (<58kg) is also a risk factor for osteoporosis. Estrogen deficiency is a risk factor for low bone density. Older age at menarche and younger age of menopause predispose women to a relative estrogen deficiency. Our study focused on women of reproductive age. Estrogen deficiency probably causes bone loss by 2 distinct mechanisms: Activation of new bone remodeling sites, and exaggeration of the imbalance between bone formation and resorption (38).

There was a notable discrepancy in the peak level of education achieved and employment between the patients and controls. In a Zimbabwean study, 43.1% of epileptics were unemployed (43). Our figures stand at 55.1%. These findings may

have an indirect influence on bone health and may also serve as a pointer to the social difficulties experienced by people living with epilepsy. The findings suggest that women living with epilepsy have a higher school drop out rate and hence a lower likelihood of subsequent employment. Smith et al showed that epilepsy patients predominantly rely on social welfare for support; only 11% are self-supporting and 62% were receiving a disability grant. His study population, however, comprised both males and females (45). This discrepancy in gainful employment may also have an impact on drug affordability among the patients, and ultimately compliance. Poor compliance may be reflected in the unacceptable seizure control achieved among the patients. The poor employment status of the patients may also have an impact on the quality of their diet. Reduced buying power of the patients may result in a reduced dietary intake of calcium and vitamin D, which impacts negatively on bone health.

A slight majority of patients were classified as having partial seizures. This encompassed the entities of Simple Partial, Complex Partial and Partial with secondary generalization. Munyoki et al, in his assessment of epilepsy in a malaria endemic region in Kenya, also found partial seizures to be the most prevalent (42). This is in contrast to studies in Zimbabwe and India where majority of the patients had Generalized seizures (43, 44). A large majority of patients were on Enzyme inducing drugs(namely Phenobarbitone, Phenytoin and Carbamazepine) and most patients were using single drug regimens to achieve control.

Seizure control was mostly inadequate, as had previously been shown by Mativo et al (45) and in a South African study (46). In our study, this may be a reflection of the availability of drugs at the hospital vis-à-vis the economic status of the patients. At KNH the prices of drugs are subsidized therefore if a drug were to be

unavailable at the hospital, the patient may not be able to buy it at a different pharmacy at a similar cost, and may therefore miss taking the drug altogether. Other possible reasons for inadequate seizure control may relate to the type of epilepsy; some seizure types are more difficult to treat. Alternatively, an epilepsy syndrome may be being managed with an inappropriate choice of drugs. Inadequate control may also be a reflection of the social stigmatization these patients encounter and their subsequent unwillingness to take drugs. They may be looking for a 'miracle cure' in alternate forms of medicine.

Duration of therapy ranged from 1 year to22 years with the majority of patients having been on drugs for 10 years or less. Duration of therapy did not significantly alter the biochemical markers of bone turnover on sub analysis.

Bone biochemical abnormalities previously described in people on medication for epilepsy include hypocalcaemia, hypophosphateamia, and elevated serum alkaline phosphatase (29, 35) In this study there was a significant reduction of calcium in patients compared with controls. In the assumed absence of confounders such as malnutrition, this reflected osteomalacia or increased bone turnover which has long been a recognized consequence of hepatic enzyme induction of vitamin D metabolism by AEDs (13-15)

There were also significantly higher values of alkaline phosphatase, urinary calcium excretion and urine calcium creatinine ratio among the patients than among controls. Serum bone alkaline phosphatase is elevated with increased osteoblastic activity and hence is a marker of bone formation. Serum bone alkaline phosphatase is also an inducible enzyme and the fact that a vast majority of patients were on enzyme inducing drugs could have pushed up the serum levels.

On the other hand, increased urinary excretion of calcium and urine calcium creatinine ratio imply an increase in osteoclastic bone resorption. These combined findings may therefore be a reflection of increased bone loss. It is also worth noting that the absolute number of patients outside the reference range for urinary excretion of calcium was significantly higher than the number of controls. These were findings in contrast to Erbayat et al who found significantly lower urinary calcium levels in children on Valproic acid and Carbamazepine than in controls (36). His reasons for this finding were not elucidated.

The serum phosphate difference, though not significant, showed a marginally higher value among the patients. This may suggest an underlying hyperparathyroidism and hypovitaminosis D as a mechanism of AED-associated bone disease in this population. Evidence exists for inhibition of the cellular response to PTH. Fetal rats treated with Phenytoin or Phenobarbitone demonstrated an impaired response to PTH. Inhibition of the bone resorptive response to PTH could lead to hypocalcaemia and hyperphosphatemia (21)

Further analysis of abnormal absolute biochemical values among patients and controls revealed a significant difference in number of patients with hypocalcaemia and hyperphosphatemia. [OR 4.6 (95%CI 1.28-17.80) and 2.8 (95%CI 1.17-5.60) respectively]

On subgroup analysis, those having Generalized seizures had higher serum levels of calcium, phosphate and alkaline phosphatase, than those with focal seizures, although this difference was not significant. Due to the small numbers, it was not possible to subanalyse the BMDs in terms of patients with Generalized compared

with Focal seizures. Farhat et al has previously shown that patients having focal seizures had a higher total body BMD than patients having generalized seizures (14) and this may be related to the use of non-enzyme inducing drugs in the management of partial seizures.

A vast majority of the patients were solely on Enzyme inducers (73.6%) compared with only one patient on a strict enzyme sparing regimen. Due to this inequality of numbers, a subgroup analysis of biochemical parameters or BMD was not done. In other studies however, an insignificant difference was found in patients taking enzyme inducing AEDs; they had a lower BMD of the spine and other sites. (14) Holloway et al suggested that the detrimental effect of the classic AED could somewhat be curtailed with enzyme sparing drugs. (37) Such findings are however at odds with those of 2 previous studies. (11, 13)

A majority of the patients were on single drug therapy (75.4%) compared to multiple therapy (in varied combinations of mostly enzyme inducers). Patients on single drug therapy had lower calcium, phosphate and urine calcium-creatinine ratio. They had higher alkaline phosphatase and urinary calcium excretion than those on multiple drugs although this difference did not achieve statistical significance. This was in contrast to one other study. Farhat et al has previously shown that patients taking single therapy had higher vitamin D levels, serum calcium and BMD of the lumbar spine than those on multiple AEDs, however these differences were not significant. The finding from his study suggested that single drug therapy was more 'bone friendly' than multiple drugs. From his study, it is not clear which drugs were being used in combination. Our finding could be explained by the fact that in management of epilepsy, single drugs are usually stepped up to maximal doses before the introduction of a new drug. The fact that

most of our patients had inadequate seizure control may also reflect on compliance. It is naturally more difficult to achieve compliance on polytherapy, so our patients on single drugs may have been more likely to comply with treatment (and be exposed to higher dosages and drug levels) and hence be more at risk of adverse drug affects.

Quantitative CT measurement of spine bone density has similar accuracy as DEXA in experienced hands (47) and this was the method of BMD assessment used in this study. The diagnosis of osteoporosis and osteopenia according to the WHO guidelines, are based on the T score. The Z score can occasionally be helpful if it is very abnormal as it suggests a secondary cause of osteoporosis (47). In this study Z score was not assessed, and this may have affected the accuracy of the results considering most of the patients (72%) were in the 20 to 29 year age bracket.

There was no significant difference in the BMD of the lumbar spine between patients and controls. Patients in fact had a higher mean BMD than controls, which is probably a chance finding. The only subject with osteopenia happened to be a control. She was a 30year old housewife with 2 children and no history of co morbidity or recent immobilization. She had never used hormonal contraception and all her biochemical parameters were normal save for a high serum calcium of 3.25mmol/L.

There was, therefore, discordance between biochemical and radiological findings in this study. Several reasons can be proposed for this discordance; Firstly, this was a small sample size and small differences may have been missed. There is also a lag time between biochemical and radiological evidence of bone loss, with biochemical differences showing earlier (as early as 3 months after the insult) whereas radiological changes are seen between 1-3 years after the insult using

DEXA (48). Biochemical markers may therefore be a more sensitive indicator of early bone changes if this lag period is considered. Souverein et al looking at risk of fractures, found the strongest association increased with a cumulative duration of exposure to AEDs of greater than 12 years (50). If his reasoning were to be imposed on our study, the fact that majority of our patients had an exposure of ≤10 years may explain the paucity of radiological difference among our subjects.

The issue of poor seizure control may also be contributory. More than half the patients had inadequate seizure control which may be attributed to poor drug compliance. With this inadequate level of compliance, obvious changes expected as early as one year into therapy may take longer to become evident, because the offending drug is not achieving sufficiently sustained serum levels to cause clinically significant side effects. It is also evident from Odula's study, that Africans seem to have better bone health than Caucasians. (31) The WHO reference ranges for the T scores that define osteopenia and osteoporosis are based on Caucasian population studies. These ranges may be too low to apply to an African population and hence what seems a normal BMD in a Caucasian, may actually be unacceptably low for an African. We therefore need to validate the T-score reference ranges for an African population.

This discordant outcome has been shown in other studies. In one paediatric study, an adverse influence on bone density was seen with sodium Valproate but not with Carbamazepine (6) even though it is generally accepted that enzyme inducing drug would give a worse adverse effect. Also, Stephen L. J. et al showed a significantly reduced bone density in post-menopausal women at the femoral neck, but not at the lumbar spine in the same group (11). In fact, at the lumbar spine, his female patients had better BMD findings than the controls. His finding brought to the fore the possibility of greater BMD differences in the appendicular (cortical bone of the

appendages) and not the axial skeleton, in post-menopausal women. In the peri-menopausal women, however, it has been suggested that the lumbar spine may be a more sensitive indicator of bone loss, which is why it was the site of choice in this study (12).

8.0 CONCLUSIONS

1. Patients on antiepileptic drugs had higher alkaline phosphatase and lower serum calcium than controls, a sign of enzyme induction as well as enhanced bone turnover respectively.

2. Patients also showed a significantly higher Urine calcium Excretion which is a marker of bone loss.

3. There was no significant difference in BMD findings between the patients and controls.

9.0 LIMITATION

1. The study was a small one and therefore may not have been able to pick up small differences in results. Those sent for BMD were fewer than those assessed with biochemical parameters.
2. It was difficult to exclude all the confounders that could influence a woman's bone health, some of which were especially difficult to assess objectively, for example, diet, exercise status and co morbidity
3. Drug dosages were not considered and may contribute to the severity of side effects.
4. Compliance was not assessed objectively by doing serum drug levels and assumptions were based on patient history and file records.

5. Newer sensitive biochemical markers of bone metabolism e.g. amino terminal propeptide of type 1 procollagen (P1NP) as a marker of bone formation, or Tartrate-resistant acid phosphatase isoform 5b (TRACP5b) as a marker of bone resorption, were not used due to cost and availability.
6. Multiple sites for BMD combining axial and appendicular bone, may have increased the sensitivity of detection of differences in BMD.
7. This being a hospital based study may limit its applicability in generalizing the conclusions and recommendations made.

10.0 RECOMMENDATIONS

1. Further studies with larger sample sizes and possibly of a longitudinal nature, and using both appendicular and axial bone sites for BMD assessment, are required in our setting to fully elucidate the impact of AEDs on bone metabolism.
2. Health care workers should be aware of the possible side effects of AEDs on bone health; especially as regards women, who are prone to age related osteoporosis.
3. Validation of BMD T-score reference ranges in an African population warrants further studies, especially as it is becoming increasingly evident that bone health in women may be inluenced by different races and lifestyles.

REFERENCES

1. Morrell MJ. Reproductive and Metabolic Disorders in Women with Epilepsy. Epilepsia 2003; **44(Suppl 4):** 11-20
2. Kwan P, Brodie MJ. Early identification of refractory Epilepsy. N Engl J Med 2000;**342**:314-319
3. LaRoche SM, Helmers SL. The new Antiepileptic Drugs. JAMA, Feb 2004; **291:5** (605-614)
4. Morrell MJ, Sarto GE, Osborne Shafer P. et al. Health issues for women with epilepsy: a descriptive survey to assess knowledge and awareness among healthcare providers. J Womens Health Gend based Med 2000;**9:**959-65
5. Valimaki M, Tiihonen M, Laitinen K. et al. Bone mineral density measured by Dual- energy x-ray absorptiometry and novel markers of bone formation and resorption in patients on AEDs. J Bone Miner Res 1994;**9**:631-7
6. Sheth R, Wesolowski C, Jacob J. et al. Effect of Carbamazepine and Valproate on bone mineral density. J Pediatr 1995;**127:**256-62
7. Chung S, Ahn C. Effects of AED therapy on bone mineral density in ambulatory epileptic children. Brain Dev 1994;**16:**382-5
8. Bogliun G, Beghi E, Crespi V. et al. Anticonvulsant drugs and bone metabolism. Acta Neurol Scand 1986;**74:**284-8
9. Dent CF, Richens A, Rowe DFJ, Stamp TC. Osteomalacia with long-term anticonvulsant therapy in epilepsy. Br Med J 1970;**4:**69-70
10. Richens A, Rowe DFJ. Disturbance of Calcium metabolism by anticonvulsant drugs. Br Med J 1970;**4:**73-76

20. Verrotti A, Greco R, Latini G. et al. Increased bone turn over in epileptic patients treated with Carbamazepine. Ann Neurol 2000;**47:**385-388

21. Koch HV, Kraft D, von Herrath D. Influence of diphenylhydantoin and Phenobarbital on intestinal calcium transport in the rat. Epilepsia 1972;**13:**829-841

22. Hahn TJ, Hendin BA, Scharp CR. et al. Serum 25-hydroxy calciferol levels and bone mass in children on chronic AED. N Engl J Med 1975;**292:**550-554

23. Vernillo AT, Rifkin BR, Hauschka PV. Phenytoin affects osteoblastic secretion from osteoblastic rat osteosarcoma. Bone 1990;**11:**309-312

24. Kruse K, Suss A, Busse M, Schneider P. Monomeric serum calcitonin and bone turnover during anticonvulsant treatment and in congenital hypothyroidism. J Pediatr 1987;**111:**57-63

25. Hahn TJ, Hendin BA, Scharp CR. et al. Effect of chronic anticonvulsant therapy on Serum 25-hydroxy-calciferol levels in adults. N Engl J Med 1972;**287:**900-904

26. Bouillon R, Raynaert J, Claes JH. et al. the effect of anticonvulsant therapy on serum levels of 25-hydroxyvitaminD, Calcium and parathyroid hormone. J Clin Endocrinol Metab 1975;**41:**1130-1135

27. Berry JL, Mawer EB, Walker DA. et al. Effect of AED therapy and exposure to sunlight on vitamin D status in institutionalized patients. In:Oxley J, Janz J, Meinardi H, eds. Antiepileptic Therapy: Chronic toxicity of Antiepileptic Drugs- New York: Raven Press, 1983:185-192

28. Hoikka V, Savolainen K, Alhava EM. et al. Osteomalacia in institutionalized epileptic patients on long term anticonvulsant therapy. Acta Neurol Scand 1981;**64:**122-131

29. O'Hare JA, Duggan B, O'Driscoll D. et al. Biochemical evidence for osteomalacia with Carbamazepine therapy. Acta Neurol Scand 1980;**62**:282-286

30. Bogliun G, Beghi E, Crespi V et al. Anticonvulsant drugs and bone metabolism. Act Neurol Scand 1986;**74**:284-288

31. Odula C, Wanjala S. Comparative study of bone mineral densitometry in women attending the Aga Khan Hospital Nairobi GOPC. Journal of Obs and Gyn of Eastern and Central Africa Aug 2004; **17 Suppl 1**: abs 53

32. Odawa F, Ojwang S, Muia Ndavi et al. The prevalence of post menopausal osteoporosis in black Kenyan women. Journal of Obs and Gyn of Eastern and Central Africa Aug 2004; **17 Suppl 1:** abs 37

33. Walker-Bone K, Arden K, Cooper C. Epidemiological aspects of osteoporosis. Reviews of contemporary pharmacotherapy 1998; **9:** 225-231

34. Cummings S, Nevitt M, Browner W. et al. Risk factors for hip fractures in white women. NEJM 1995;**332:**767-773

35. Gough H, Goggin T, Bissessar A. et al. A comparative study of the relative influence of different anticonvulsant drugs vs. exposure and diet on vitamin D and calcium metabolism in outpatients with epilepsy. QJM(New series 59) 1986;**230**:569-77

36. Erbayat Altay E, Serdaroglu A, Tumer L. et al. Evaluation of bone mineral metabolism in children receiving Carbamazepine and Valproic acid. J Pediatr Endocrinol Metab 2000;**13:**933-939

37. Holloway L, Paulson A, Seale C. et al. Skeletal status of women with epilepsy. J Bone Miner Res 2000; **15(suppl 1):** SA 302. Abstract.

38. Kasper D, Hauser S, Braunwald E, et al.(editors) Harrison's principles of internal medicine 16th edition Vol II;333: 2271

39. Hahn T. Bone complications of anticonvulsants. Drugs 1976;**12:**201-211

40. Perucca E, Hedges A, Makki K et al. A comparative study of the relative enzyme inducing properties of anticonvulsant drugs in epileptic patients. British Journal of Clinical Pharmacology 1984;**18:**401-410

41. Harrington M.G, Hodkinson H.M. Anticonvulsants and bone disease in the elderly. Journal of the Royal Society of Medicine 1987;**80:**425-427

42. Munyoki G.M, Kwasa T.O, Newton C.R, Amayo E.O. The prevalence of epilepsy in a malaria endemic region of Kenya. MMed dissertation, University of Nairobi, 2005.

43. Sebit MB, Mielke J. Epilepsy in sub-Saharan Africa: its socio-demography, aetiology, diagnosis and EEG characteristics in Harare, Zimbabwe. East African Medical Journal 2005;**82(3):**128-137

44. Ray BK, Bhattacharya S, Kundu TN. et al. Epidemiology of Epilepsy-Indian perspective. Journal of Indian Medical Association 2002 May;**100(5):**322-6

45. Mativo P.M, Amayo E.O, Oyatsi D, Jowi J.O. Factors associated with poor control of epilepsy at Kenyatta National Hospital adult neurology clinic. MMed Dissertation, University of Nairobi 2004.

46. Smith JL, Sacks E. Epilepsy in a community; Management and social impact. South African Medical Journal 1985 Jun;**67(24):**981-4

47. Henderson KE, Baranski TJ, Bickel PE. The Washington Manual endocrinology subspecialty consult.;**2005 edition**: 138-154

48. Endres DB, Rude RK. Mineral and bone metabolism in Tietz textbook of clinical chemistry 3rd edition; Burtis CA, Ashwood RE (Ed); W B Saunders Company 2005: 1936

49. Wingo P.A, Higgins J.E, Rubin G.L, Zahniser S.C. An Epidemiological study approval to reproductive health, WHO/HRP/EPI/1994 pg 167

50. Souvrein PC, Webb DJ, Weil JG. et al. Use of anti epileptic drugs and risk of fractures : Case control study among patients with epilepsy. Neurology 2006 may;**66:**1318-1324

APPENDICES

APPENDIX I

CONSENT FORM

I..... of ..... hereby agree to participate in the study on 'Bone Metabolism in ambulatory women of reproductive health using antiepileptic drugs', being undertaken by Dr.Judith Kwasa. I understand the nature of the study and that my participation in this study is on voluntary basis and have willingly agreed to take part in it.

Signed.....
Patient/Parent/Guardian

Witness.....
Date.....

ASSENT FORM

(For Subjects less than 18 years of age)

I..... of ..... being under the age of 18 and with consent from my parent/guardian, hereby agree to participate in the study on 'Bone Metabolism in ambulatory women of reproductive health using antiepileptic drugs', being undertaken by Dr.Judith Kwasa. I understand the nature of the study and that my participation in this study is on voluntary basis and have willingly agreed to take part in it.

Signed.....
Patient

Witness.....
Date.....

RADIOLOGY CONSENT FORM

(For use by those going for BMD)

I..... of ..... hereby agree to undergo a radiological test as part of the requirements in the study on 'Bone Metabolism in ambulatory women of reproductive health', being undertaken by Dr.Judith Kwasa. I understand that this involves exposure to a minimal amount of radiation, and that my participation in this study is on voluntary basis and I have willingly agreed to take part in it.

Signed.....
Patient/Parent/Guardian

Witness.....
Date.....

APPENDIX II

CONSENT EXPLANATION

My name is Dr Judith K Kwasa and I wish to conduct a study comparing Bone Mineral Density in 2 groups of women of reproductive age: Those on antiepileptic drugs and those not on such drugs. This study is in part fulfilment of my postgraduate programme in Internal medicine at the Departmant of Medicine, University of Nairobi, and has been approved by the KNH Scientific and Ethical review committee.

The aim of the study is to assess the effects of long term antiepileptic drug therapy on bone health, by comparing various clinical parameters in two groups of females: One group having used antiepileptic drugs for more than a year, and the other not having used the drugs at all.

You have been requested to voluntarily participate in this study because you fit the inclusion criteria. By participating, you will know your baseline bone metabolism levels after the various tests are carried out, at no cost to yourself.
As the patient, you have the right to choose to participate in the study or not. If you decline to participate in the study, it will in no way affect the standard of medical care that you will continue to receive. You also have the right to withdraw from the study for whatever reason at any time.

The study involves:

- Taking a history and performing a physical examination to identify any other cause of disease.
- Taking urine sample for Urine calcium Creatinine ratio and calcium excretion.
- Taking blood for serum calcium, phosphate and alkaline phosphatase levels.
- The possibility of a bone mineral density evaluation. This is a radiographical test used to assess the density of your bone structure.

The people who agree to participate in the study will be required to sign the consent form. For people under 18 years of age, this information will be given to their parents/guardians and they too will be required to give a signed assent.

All results shall be communicated to you and your primary doctor and appropriate management instituted where need be. At the end of the study the results will be presented to the Internal medicine department. Confidentiality will be ensured.

Any further questions can be directed to me, Dr Judith Kwasa or my principal supervisor Dr Thomas Kwasa, Department of Medicine, University of Nairobi Tel 2726300

APPENDIX III

SCREENING QUESTIONAIRE

Date of Screening:.....
Name:.....
Date of Birth.....
Age (Y):.....

Subject Source (Tick One)

1) Patient
2) Patient Escort
3) Student
4) Staff
5) Other (Specify)

Are you known to have/be (Y/N)

1) Pregnant or 6 weeks amenorrhea
2) Severe Renal Disease
3) Liver Disease (excluding viral hepatitis)
4) Cardiac Disease
5) Thyroid/Parathyroid Disease
6) Cancer of any kind
7) Bedridden for 2 weeks over the last month e.g. for a fracture
8) Chronic Diarrhoea (2 weeks) or malabsorption
9) Hormonal contraception over last 1 year
10) Using any of the following: Vit D or Calcium supplements
    Steroids
    Thiazide Diuretics

(If Y for any of the above, please exclude subject from study participation and screen another subject from the same subject source)

APPENDIX IV: STUDY PROFORMA

Study Number:.....
Date:.....
Name
Hospital Number (Patients):.....
DOB:.....
Age (Y):.....
Contact (Tel):.....

Demographics

Marital Status

1) Single
2) Married
3) Separated
4) Widowed
5) Divorced

Usual Residence:.....

Usual Occupation

1) Employed
2) Unemployed
3) Never had formal employment
4) Retired

Level of formal education

1) None
2) Primary
3) Secondary
4) Tertiary

Epilepsy History (Patients only)

Date of Diagnosis of Epilepsy

Seizure type (Tick One)

1) Partial
2) Complex Partial
3) Partial with secondary generalisation
4) *Generalised Tonic Clonic*
5) Other (Specify)

Seizure Frequency (Tick One)

How often do you have fits while on regular medication?

1) < or = once every 6 months
2) >once every 6 months

Seizure control From Patient File (Tick one)

How often are seizures documented?

1. < or =Once every 6 months
2. >once every 6 months

Epilepsy drugs (Start with most recent medications)

Drug	Daily Dosage	Duration of use
1		
2		
3		
4		
5		
6		
7		
8		
9		

Possible cause of epilepsy (Tick wherever applicable)

1) Post traumatic
2) Familial
3) Post CVA
4) Unknown

Family History

Did or do any of your relatives suffer from: (Y/N)

1) Hypertension
2) Diabetes
3) Kidney Disease
4) Epilepsy
5) Stroke

If "Y" please specify relationship

1) Father/Mother
2) Brother/Sister
3) Child
4) Other (Please Specify)

Social History

Do you take any alcohol? (Y/N)
If "Y" please quantify weekly intake: bottles of beer/wk or shots/wk:.....
For how long? .....
Do you smoke? (Y/N)
If "Y" please quantify daily consumption cigarettes per day:.....
For how long? .....

Ob/Gyn History

Age at Menarche:.....
Parity:.....
LMP .....
Have your last 3 menses been regular? (Y/N)
If not, please give last 3 cycle dates.....
.....
Any previous history of: (Y/N)
1) Hysterectomy
2) Oophrectomy
3) Amenorrhea NOT DUE to pregnancy

Contraception (Y/N)
If "Y" please specify method used: .....
.....

PHYSICAL EXAMINATION

BP:...../.....
PR.....bpm
Temp.....C
Weight (kg).....
Height (cm).....
Oedema.....
Jaundice.....

Social History

Do you take any alcohol? (Y/N)

If "Y" please quantify weekly intake: bottles of beer/wk or shots/wk:.....

For how long? .....

Do you smoke? (Y/N)

If "Y" please quantify daily consumption cigarettes per day:.....

For how long? .....

Ob/Gyn History

Age at Menarche:.....

Parity:.....

LMP .....

Have your last 3 menses been regular? (Y/N)

If not, please give last 3 cycle dates.....

.....

Any previous history of: (Y/N)

1) Hysterectomy
2) Oophrectomy
3) Amenorrhea NOT DUE to pregnancy

Contraception (Y/N)

If "Y" please specify method used: .....

.....

PHYSICAL EXAMINATION

BP:...../.....

PR.....bpm

Temp.....C

Weight (kg).....

Height (cm).....

Oedema.....

Jaundice.....

Please answer "Y" or "N" for the following: If "Y" please specify

	Y	N
Neck: Thryroid Enlargement?		
CVS: Any abnormality?		
PA: Any abnormality?		

If Y, please specify: .....

.....

.....

.....

.....