# CHAPTER III

# RESEARCH METHODOLOGY

## 3.1 Research Questions

### 3.1.1 Primary Research Question

Does long-term antiepileptic drug (AED) therapy have a significant adverse effect on BMD in Thai ambulatory pre-menopausal epileptic patients in comparison with age range-matched healthy Thai pre-menopausal females?

### 3.1.2 Secondary Research Questions

3.1.2.1 Is there any difference in BMD between long-term AED monotherapy and polytherapy in Thai ambulatory pre-menopausal epileptic patients?

3.1.2.2 Is there any difference in BMD between long-term CYP 450 inducing and non-inducing AED therapy in Thai ambulatory pre-menopausal epileptic patients?

3.1.2.3 Is there correlation between duration of AED therapy and BMD?

3.1.2.4 Is there any difference in BMD between patients with localization-related epilepsy and generalized epilepsy who have taken AEDs 3 years or more?

## 3.2 Research Objectives

### 3.2.1 Primary Objective

To study whether there is significant adverse effect of long-term AED therapy on BMD in Thai ambulatory pre-menopausal epileptic patients in comparison with age range-matched healthy Thai pre-menopausal females.

3.2.2 Secondary Objectives

3.2.2.1 To compare the adverse effect on BMD between long-term AED monotherapy and polytherapy in Thai ambulatory pre-menopausal epileptic patients.

3.2.2.2 To compare the adverse effect on BMD between long-term cytochrome P 450 inducing and non-inducing AED therapy in Thai ambulatory pre-menopausal epileptic patients.

3.2.2.3 To assess the relationship between duration of AED therapy and BMD.

3.2.2.4 To compare the difference of BMD between patients with localization-related and generalized epilepsy who have taken AEDs 3 years or more.

## 3.3 Hypothesis

3.3.1 Research Hypothesis

Long-term AED therapy had significant adverse effect on BMD in Thai ambulatory pre-menopausal epileptic patients in comparison with age range-matched healthy Thai pre-menopausal females.

3.3.2 Statistical hypothesis

According to the primary research question:

Null hypothesis ($H_0$) stated that long-term AED therapy had no significant adverse effect on BMD in Thai ambulatory pre-menopausal epileptic patients in comparison with age range-matched healthy Thai pre-menopausal females.

Alternative hypothesis ($H_A$) stated that long-term AED therapy had significant adverse effect on BMD in Thai ambulatory pre-menopausal epileptic patients in comparison with age range-matched healthy Thai pre-menopausal females.

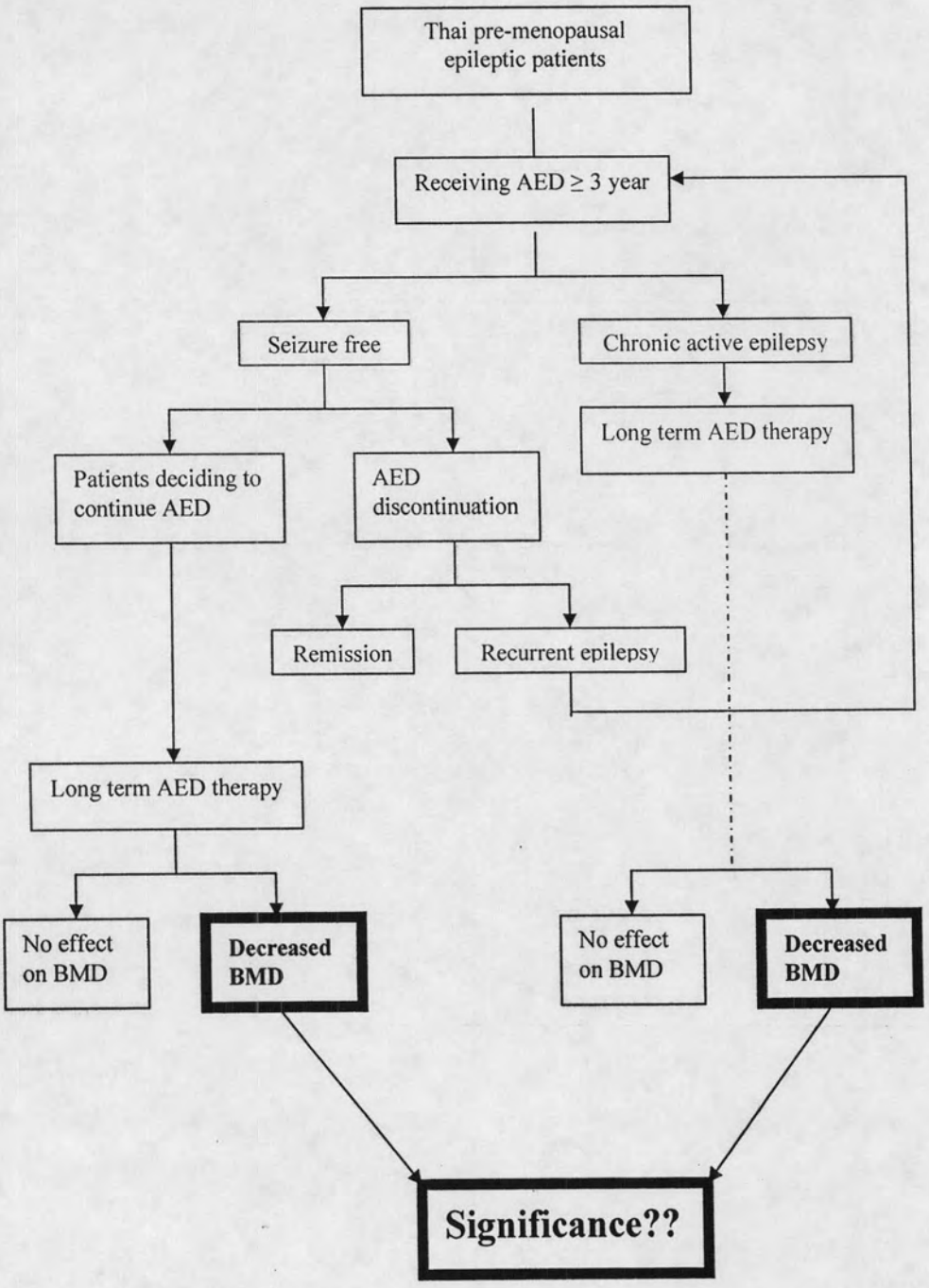
$$H_0 : \mu_1 = \mu_2$$

$$H_A : \mu_1 \neq \mu_2$$

where $\mu_1$ = the mean of BMD in Thai ambulatory pre-menopausal female epileptic patients who have been on AEDs for at least 3 years

$\mu_2$ = the mean of BMD in age range-matched healthy Thai pre-menopausal females

## 3.4 Conceptual Framework




AED : antiepileptic drug

BMD : bone mineral density

## 3.5 Fundamental Agreement

There is no fundamental agreement in the study.

## 3.6 Key Words

Bone mineral density (BMD), antiepileptic drug (AED), pre-menopausal, epilepsy

## 3.7 Operational Definitions

- Thai female is defined by a woman whose all father, mother, paternal and maternal grand father and grand mother (three generations) were all Thai or Chinese and all were born in Thailand.
- Long-term AED therapy is defined by the continuous use of one or more AEDs for 3 years or more (7).
- Age range-matched healthy control is defined by pre-menopausal healthy female aged within the same decade as epileptic patients (20-30, 31-40, 41-50 years).
- Significant level of adverse effect on BMD from long-term AED therapy is defined by 10% decrement of BMD as observed in the previous studies (14, 15).
- AED non-compliance is defined by researcher by history of missing AED more than 30% of prescription for the last 3 years.
- Monotherapy cytochrome P 450 enzyme inducing AED and polytherapy AEDs with one cytochrome P 450 enzyme inducing AED are classified as cytochrome P 450 enzyme inducing AED group.
- Monotherapy cytochrome P 450 enzyme non-inducing AED and polytherapy AEDs without cytochrome P 450 enzyme inducing AED are classified as cytochrome P 450 enzyme non-inducing AED group.
- Pre-menopausal female means women who still have active menstruation and serum estradiol > 5 pg/ml.

- Active menstruation means women who still have menstruation and never miss menstruation continuously longer than 6 months except pregnant period.
- Chronic diseases affecting bone metabolism or homeostasis of calcium or phosphorus including
  - ❖ History of thyroid diseases including any conditions that cause overt symptomatic hyperthyroidism with laboratory thyroid function test (serum T3, Free T4 and TSH) confirmation
  - ❖ History of renal diseases including any conditions that cause renal tubular dysfunction, i.e.
    - O Chronic kidney disease screened by serum creatinine 1.5 mg/dl or more
    - O Proteinuria screened by urine protein positive 1+ or more
    - O Nephritic syndrome screened by urine white or red blood cell more than 5 cells/high power field
    - O Renal tubular acidosis screened by normal anion gap metabolic acidosis
  - ❖ History of hepatic diseases including any conditions that affect vitamin D metabolism, i.e.
    - O Hepatomegaly by physical examination
    - O Abnormal liver function test by any of the followings:
      - ■ Serum albumin less than 3.5 g/dl
      - ■ Serum total or direct bilirubin more than upper normal limit (1.2 mg/dl or 0.5 mg/dl respectively)
      - ■ Serum AST (SGOT) or ALT (SGPT) more than three times of upper normal value (40 U/l), that is 120 U/l or more
      - ■ Serum alkaline phosphatase more than two times of upper normal value (117 U/l), that is 234 U/l or more
  - ❖ History of small bowel disorders including any conditions that can disturb calcium absorption screened by any following conditions.

- Previous gastric or small bowel resection or gastrointestinal bypass surgery
- History of passing stool more than 3 times a day continuously for one month during last 3 years
- History of passing oil in stool continuously for one month during the last 3 years
- Physical signs of malnutrition, i.e. pale, koilonychia, glossitis, cheilosis, stomatitis, edema, ascites, low body mass index less than 18.5 $kg/m^2$

- History of receiving drugs or substances affecting bone metabolism or homeostasis of calcium or phosphorus continuously more than six months during the last 3 years, i.e. calcium, vitamin D, thyroid hormone, steroid substance, sex hormones other than for contraception indication, calcitonin, bisphosphonate, parathyroid hormone, diuretics.
- Underweight, a risk factor of decreased BMD, is defined by body mass index (BMI) less than 18.5 $kg/m^2$ (27).
- The severity of bone loss is categorized relative to mean BMD (BMD) in young, healthy women. A 1-unit change in T-score corresponds to a 1 standard deviation difference from the reference population. World Health Organization operational definition for normal BMD is within 1 SD of a young normal. Osteopenia is between 1 and 2.5 SD and osteoporosis is 2.5 SD below that of a young normal adult (21, 28, 29).
- BMD is measured by dual energy X-ray absorptiometry (DXA) that is recommended by most guidelines as the preferred technique for measurement of BMD (12, 30-32). BMD in the study uses a GE Medical Systems LUNAR Prodigy DF+15974 densitometer at Division of Endocrinology and Metabolism, Faculty of Medicine, Siriraj Hospital. The precision of this machine, expressed as the coefficient of variance (CV), is 0.08%.

- BMD measurement is reported as $g/cm^2$ (measured value), T-score (the difference in standard deviation units between the measured BMD value and the peak bone density in the normal reference population) and Z-score (the standard deviation units from age-sex-specific reference score). Reference score is from Japanese people.

## 3.8 Research Design

As the study risk factor (AEDs) was not allocated to the study population, the research was an observational study. In addition, it was designed to compare the results of the epileptic patients who exposed to the study risk factor (AEDs) with that of the control group (age range-matched healthy pre-menopausal females), therefore, it was an analytical study. Furthermore, data of study group was planned to collect at one point of time when they had already been on AED for 3 years or more. Thus, it was a cross sectional study.

As a result, the research was designed as **a cross sectional, analytical, observational study** of long term AED adverse effect on BMD in Thai pre-menopausal epileptic patients.

The study design was appropriate for preliminary examining problem of AED on BMD since it was feasible to perform in an acceptable duration of the study and was able to shed a light for this problem.

## 3.9 Research Methodology

### 3.9.1 Population

#### 3.9.1.1 Target population

The target population was Thai ambulatory pre-menopausal epileptic patients receiving single or multiple AEDs for 3 years or more.

3.9.1.2 Study population

The study population was Thai ambulatory pre-menopausal epileptic patients receiving single or multiple AEDs for 3 years or more treated in epilepsy clinic at Siriraj Hospital.

Siriraj Hospital was selected for the study site as there were many chronic active epileptic patients attending this epilepsy clinic. It was also feasible for all measurement that were done in Siriraj Hospital since researcher was working in Siriraj. However, the majority of epileptic patients may live in the central part of Thailand that might not represent epileptic patients in other part of Thailand.

### 3.9.2 Inclusion criteria for study population

The study population with all of the following criteria was recruited:

3.5.3.1 Thai ethnic female

3.5.3.2 Aged between 20 - 50 years

3.5.3.3 Having active menstruation

3.5.3.4 Having epilepsy for 3 years or more

3.5.3.5 Regularly receiving single or multiple AEDs for 3 years or more

3.5.3.6 Able to walk without walking aids.

3.5.3.7 Totally independent on doing daily activity.

### 3.9.3 Exclusion criteria for study population

If the eligible epileptic patients had the following conditions, they were then excluded:

3.9.3.1 Not AEDs compliance

3.9.3.2 Having other chronic diseases affecting bone metabolism or homeostasis of calcium or phosphorus during the last 3 years including hyperthyroidism, renal diseases, hepatic diseases,

gastrointestinal disorders affecting calcium, phosphorus and vitamin D absorption

3.9.3.3 Currently using or ever receiving drugs or substances affecting bone metabolism or homeostasis of calcium or phosphorus continuously more than six months during the last 3 years, i.e. calcium, vitamin D, thyroid hormone, steroid substance, sex hormones other than for contraception indication, calcitonin, bisphosphonate, parathyroid hormone and diuretics

3.9.3.4 Having other conditions affecting bone metabolism or homeostasis of calcium or phosphorus or BMD during the last 3 years: post menopausal period, amenorrhea, underweight (body mass index less than 18.5 $kg/m^2$), being pregnant, being in lactation period, history of bone fracture at study BMD sites

3.9.3.5 Not provide consent form

### 3.9.4 Matched control group criteria

Thai healthy women were asked to participate if they had all the following criteria.

3.9.4.1 Pre-menopausal female, having active menstruation.

3.9.4.2 Aged within the same range as the recruited epileptic patients (20-30, 31-40, 41-50 years).

3.9.4.3 With the same economic status as the recruited epileptic patients.

3.9.4.4 Not having the exclusion criteria as the study patients

3.9.4.5 Providing consent form

### 3.9.5 Sample Size

As the study was a cross sectional, analytical, observational study to compare BMD of the epileptic patients receiving long-term AEDs and that of the age range-matched healthy subjects, sample size estimation was then based on the comparison of two independent means.

Values of mean BMD and its standard deviation among healthy Thai subjects were obtained from a study in rural Thai adults in Khon Kaen province as shown in Table 2 (10).

Table 2 Bone mineral density (BMD) among rural Thai adults in Khon Kaen province

| Bone site | BMD ($g/cm^2$) : mean ± SD | | |
|---|---|---|---|
| | Pre-menopausal women (n = 117) | Post- menopausal women (n = 135) | Difference (%) |
| Distal radius | 0.69±0.06 | 0.54±0.11 | 21.7 |
| Femoral neck | 1.00±0.13 | 0.75±0.16 | 25.0 |
| Lumbar spine | 1.15±0.13 | 0.88±0.20 | 23.5 |

Table 2 reveals that the differences in mean BMD at three common bone fracture sites i.e., distal radius, femoral neck and lumbar spine between 117 pre-menopausal women and 135 post-menopausal women were 21.7%, 25.0% and 23.5% respectively. With the aim of having bone mass close to that in pre-menopausal healthy women, a 10% difference in mean BMD between Thai pre-menopausal epileptic patients receiving AEDs 3 years or more and age-matched healthy Thai pre-menopausal controls was considered to be a clinically significant difference. Since sample size determination was based on an absolute difference in mean BMD between two groups, expected absolute difference at each bone site and its SD were displayed in Table 3.

Table 3 Estimated difference in bone mineral density (BMD) between pre-menopausal healthy females and pre-menopausal epileptic patients receiving antiepileptic drugs (AEDs)

| Bone site | Estimated BMD ($g/cm^2$) with expectation of 10% difference between the two groups : mean ± SD | | |
|---|---|---|---|
| | Pre-menopausal healthy females | Pre-menopausal epileptic patients receiving AEDs | Absolute difference |
| Distal radius | 0.69 ± 0.06 | 0.62 ± 0.06 | 0.07 ± 0.06 |
| Femoral neck | 1.00 ± 0.13 | 0.90 ± 0.13 | 0.10 ± 0.13 |
| Lumbar spine | 1.15 ± 0.13 | 1.04 ± 0.13 | 0.11 ± 0.13 |

Among the three bone sites, the difference in mean of BMD between the two groups was smallest for distal radius i.e., 0.07 $g/cm^2$, whereas the standard deviation of BMD was largest for femoral neck and lumbar spine i.e., 0.13 $g/cm^2$. Thus, to be on a safe side, the minimum absolute difference in mean of BMD of 0.07 $g/cm^2$ and the maximum standard deviation of 0.13 $g/cm^2$ were used in sample size calculation as shown in the following details.

n for each group = $2\sigma^2 [Z_{\alpha/2} + Z_{\beta}]^2 / [\bar{X}_1 - \bar{X}_2]^2$

where $\alpha$ = Probability of type I error = 0.05 (2-tailed)

$Z_{0.025}$ = 1.96

$\beta$ = Probability of type II error = 0.20

$Z_{0.2}$ = 0.842

$\bar{X}_1$ = Expected mean BMD among healthy pre-menopausal subjects = 0.69

$\bar{X}_2$ = Expected mean BMD among pre-menopausal epileptic patients receiving AEDs = 0.62

$\sigma$ = Standard deviation of BMD = 0.13

Therefore, n for each group = $2 (0.13)^2 [1.96 + 0.842]^2 / (0.07)^2$

= 54.16 $\simeq$ 55 persons

### 3.9.6 Measurement Methods

All measurements were done at Siriraj Hospital.

3.9.6.1 Bone mineral density

BMD was measured by dual energy X-ray absorptiometry (DXA) using a GE Medical Systems LUNAR Prodigy DF+15974 densitometer at Division of Endocrinology and Metabolism, Faculty of Medicine, Siriraj Hospital. The precision of this machine, expressed as the coefficient of variance (CV), is 0.08%. The machine had been calibrated before using every day. It passed the precision scale every time.

3.9.6.2 Other variables were measured by appropriate laboratory tests at the laboratory center of Department of Clinical Pathology, Siriraj Hospital.

Table 4 Method and machine used for assay serum level of variables and urine protein

| Test | Method | Machine |
|---|---|---|
| Total calcium | Enzymatic colorimetry | Modula P 800 |
| Ionized calcium | Ion selective electrode | Modula ISE 1800 |
| Phosphorus | Enzymatic colorimetry | Modula P 800 |
| Parathyroid hormone | Electro chmiluminascnce T4 | Modula E 170 |
| T3 | Electro chmiluminascnce T4 | Modula E 170 |
| Free T4 | Electro chmiluminascnce T4 | Modula E 170 |
| Thyroid stimulating hormone (TSH) | Electro chmiluminascnce T4 | Modula E 170 |
| Estradiol (estrogen) | Electro chmiluminascnce T4 | Modula E 170 |
| Creatinine | Enzymatic colorimetry | Modula P 800 |
| Sodium | Ion selective electrode | Modula ISE 1800 |
| Potassium | Ion selective electrode | Modula ISE 1800 |
| Chloride | Ion selective electrode | Modula ISE 1800 |
| Bicarbonate | Enzymatic colorimetry | Modula P 800 |
| Albumin | Enzymatic colorimetry | Modula P 800 |
| Total bilirubin | Enzymatic colorimetry | Modula P 800 |
| Direct bilirubin | Enzymatic colorimetry | Modula P 800 |
| AST (SGOT) | Enzymatic colorimetry | Modula P 800 |
| ALT (SGPT) | Enzymatic colorimetry | Modula P 800 |
| Alkaline phosphatase | Enzymatic colorimetry | Modula P 800 |
| Urine protein | Reagent strip | Menibron M |

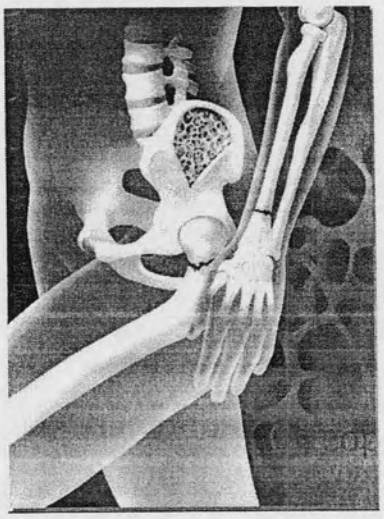
### 3.9.7 Study Procedure

All epileptic patients treated in the epilepsy clinic at Siriraj Hospital were structurally interviewed with a case record form (Appendix D) by the researcher or a research nurse for baseline variables including date of interview, date of birth, weight, height, menstruation, ability to walk, ability to perform daily activity (totally independent or dependent), duration of epilepsy, type of epilepsy, current and the past AEDs use, AED compliance, duration of AED therapy. Use of other medication or substance, other chronic diseases e.g. thyroid, renal, hepatic, gastrointestinal diseases were also collected for later reviewing for effects on bone metabolism or homeostasis of calcium or phosphorus. General physical examination was performed by the researcher.

After interview and physical examination, all eligible epileptic patients' blood sample were collected for assessment of baseline characteristics, i.e. serum level of creatinine, sodium, potassium, chloride, bicarbonate, albumin, total and direct bilirubin, AST (SGOT), ALT (SGPT), alkaline phosphatase, thyroid function test (T3, Free T4, TSH), parathyroid hormone, estrogen. Urine examination was also performed. Then included participants' BMD at three bone sites (figure 1: lumbar, left femur and left radius) was measured by dual energy X-ray absorptiometry (DXA) using GE LUNAR densitometer at Siriraj Hospital.

Age range-matched healthy pre-menopausal controls in the same socioeconomic status as patients were asked for participation. They were interviewed, general physical examined and had all measurement done as the study patients. In addition, blood was assayed for total calcium, ionized calcium and phosphorus. If they are abnormal, the participated controls will be excluded.

Figure 1 Bone site for BMD measurement



### 3.9.8 Data Collection

#### 3.9.8.1 Demographic data and baseline characteristics

The researcher or a research assistant collected demographic data and baseline characteristics as the follows by using the case record form (Appendix D):

- Baseline data

For patients only:

Duration of epilepsy (month), type of epilepsy (localization-related epilepsy, generalized epilepsy, unclassified epilepsy), AED (number, type, duration, compliance)

For both patients and controls:

Age (year), weight (kilogram), height (meter), body mass index (kilogram/meter$^2$), menstruation (currently active / history of amenorrhea more than 6 months), ability to walk without walking aids, ability to perform daily activity (independent / dependent), use of other medication or substance affecting bone and calcium metabolism, other chronic disease affecting bone and calcium metabolism including thyroid diseases, renal diseases, hepatic diseases, small intestine disorders

- **Laboratory tests**

Level of serum creatinine, sodium, potassium, chloride, bicarbonate, albumin, total bilirubin, direct bilirubin, AST (SGOT), ALT (SGOT), alkaline phosphatase, total calcium,

ionized calcium, phosphorus, T3, free T4, TSH, estradiol (E2), parathyroid hormone, Beta-cross Laps (Pyrinidoline cross-linked carboxy-terminal telopeptide of type I collagen), P1NP (procollagen procollagen type 1 amino-terminal propeptide), Osteocalcin (N-MID) and urine examination

3.9.8.2 Outcome

BMD ($g/cm^2$, Z-score, T-score) was performed at 3 sites

- Lumbar (L) 2-4
- Left femur (neck, trochanter, total)
- Left radius (radius UD, Radius 33%)

### 3.9.9 Data Analysis

Baseline characteristics were screened and the included participants in both patient and control groups were then excluded regarding to the following exclusion criteria.

- Low body mass index by BMI < 18.5 $kg/m^2$
- Hyperthyroidism by T3 more than upper limit 180.00 ng/dl, free T4 more than upper limit 1.900 ng/dl, TSH less than lower limit 0.23 uU/ml
- Renal disease by creatinine more than upper limit 1.5 mg/dl, urine protein 1+ or more, urine white or red blood cell more than 5 cells/high power field, renal tubular acidosis screened by normal anion gap metabolic acidosis blood (pH < 7.4 and sodium-chloride-bicarbonate > 12)
- Hepatic disease by albumin less than lower limit 3.5 g/dl, total or direct bilirubin more than upper normal limit (1.2 mg/dl or 0.5 mg/dl respectively), SGOT or SGPT more than three times of upper normal value (>120 U/l), alkaline phosphatase more than two times of upper normal value (> 234 U/l)
- Post menopausal status by estradiol less than lower normal 5 pg/ml

For the control group, other baseline characteristics were further screened and excluded regarding to the exclusion criteria, i.e. total calcium outside normal range 8.1-10.4 mg/dl, ionized calcium outside normal range 4.6-5.2 mg/dl, phosphorus outside normal range 2.2-5.0 mg/dl, parathyroid hormone outside normal range 15.00-65.00 pg/ml.

Characteristics of study subjects were described and compared between the patient and control groups using (1) mean, standard deviation (SD), median, minimum and maximum for quantitative data e.g., age, body mass index, duration of AEDs receiving and (2) frequency for qualitative data e.g., number of participants in each age range group, type of epilepsy, type of AEDs and number of AEDs.

To compare the primary outcome, BMD ($g/cm^2$, Z-score, T-score), at the 3 bone-sites, i.e. lumbar 2-4, left femur (neck, trochanter, total) and left radius (radius UD, Radius 33%), between the epileptic patient and control groups, unpaired t-test was employed. Mean difference and its 95% confidence interval (CI) were reported along with p-value. As a supporting analysis, BMD in the epileptic patient and healthy control group was also categorized into 2 groups i.e., normal and abnormal BMD, i.e. osteopenia, osteoporosis (29). Chi-square test was used to compare the proportion of abnormal BMD between the two groups.

To test the difference in BMD between epileptic patients receiving long-term AED monotherapy and polytherapy, unpaired t-test was applied.

Similarly, to assess the difference in BMD between epileptic patients receiving long-term CYP 450 inducing AEDs and non-inducing AEDs, Mann-Whitney U test was employed.

To assess the relationship between duration of AEDs therapy and BMD in epileptic patients that was not normal distribution, Spearman rank correlation was applied. Correlation coefficient with p-value was reported.

To compare the difference of BMD between patients with localization-related and generalized epilepsy who had taken AEDs 3 years or more. Unpaired t-test was

employed. Mean difference and its 95% confidence interval (CI) were reported along with p-value.

All statistical analysis was performed using SPSS version 16.0. Two-sided p-value of less than 0.05 was considered statistically significant.

### 3.9.10 Ethical Considerations

Prior to conducting a time-consumed prospective study of long term AED effect on BMD, a cross sectional analytical observational study design would be appropriate for a preliminary documentation of this clinical problem. The study design obtained some conclusive results.

For BMD measurement by dual energy X-ray absorptiometry at three bone sites, the amount of radiation exposure was approximately equal to a chest x-ray. Therefore, this BMD measurement technique had no clinical significant risk. Venous puncture for blood tests and urine examination were routine medical tests and carried no risk. For the convenience of the patients, all study procedure was completely carried on in one day.

To ensure that all eligible patients and age range-matched healthy controls feeled free to participate in the study, they were invited by a trained research assistant, not by their doctors, to participate in the study. They were thoroughly explained the detail of study procedure, amount of blood sample and urine collection, risk and safety of BMD measurement technique. In addition, they were explained about the usefulness of the results for themselves and for other epileptic patients in regarding to recommendation of prevention measures for adverse effects of AEDs on BMD.

Overall, there was no harm whereas there was benefit to all participants. Participants' confidentiality was exercised by recording their data in a case record form with code identifiable and accessible by only researchers. Consent form was obtained.

All participants did not have to pay for any study procedure and did not receive money or any gift for participation except for transport fee, 200 baht.

The research proposal, the study procedure, the information on risk and safety of BMD measurement technique and the consent form were in accordance with the

**Helsinki Declaration.** In addition, all were approved by the Ethic Committee at Siriraj Hospital and at Chulalongkorn University.

After approval by Siriraj Ethic Committee, the research proposal was submitted for research grant at Siriraj Hospital.

### 3.9.11 Limitation

BMD change is a slow process and is affected by many factors. In order to evaluate the adverse effect of AEDs on BMD, a prospective cohort study design comparing BMD before and after receiving AEDs periodically with an appropriate reference BMD would be more reliable and more valuable. However, for a feasibility study, a cross sectional, analytical observational study design is more appropriate for the current situation.

BMD is affected by several factors including ethnicity. There is no reference for BMD for Thai ethnic yet. Although the use of Japan reference is considered most reasonable as Thai ethnic and Japanese ethnic are quite close. However, factors influent on BMD such as life style, food, weight bearing activity, sunlight exposure and others may be different among Thai and Japanese people. Therefore, it may not be adequately accurate to use Japan reference of BMD in the study.

Since the significant rate of BMD decrement by AEDs is not known, a 10% difference of mean BMD between epileptic patients receiving AEDs 3 years or more and healthy controls may be not adequately sensitive to explore the negative effect of AED on BMD. However, it is also not known whether the lesser difference value such as 5% will be clinically significant or not.

Most of the epileptic patients in the study are difficult to treat and the dosage and type of their AEDs are adjusted or changed throughout in order to control both their seizures and drug's side effects. Therefore, the cross sectional, analytical observational study design may be confounded by the treatment regime.

To classify the use of polytherapy AEDs with one cytochrome P 450 enzyme inducing AED as the group of cytochrome P 450 enzyme inducing AED may not be

precise. The treatment regime is adjusted or changed all the treatment period. Some treatment period, the epileptic patients may not received cytochrome P 450 enzyme inducing AED. Since there is drug to drug interaction, it is not known whether the use of polytherapy with one cytochrome P 450 enzyme inducing AED will have an effect on BMD as same as the use of polytherapy with several cytochrome P 450 enzyme inducing drug or the use of monotherapy cytochrome P 450 enzyme inducing drug.

The study do not design to control other confounding factors on BMD such as amount of sunlight exposure, vitamin D intake, calcium intake, weight bearing activity because it is very difficult and not feasible. These confounding factors may enhance or lessen the effect on BMD of AED. The control of socioeconomic status may partially balance these confounding factors.

The study recruits normal control from Siriraj Hospital medical personnel. These medical personnel may be different from the epileptic patients in the factors of environment and life style that carry some effect on BMD. It is failed to recruit normal controls that live in the same house or area of the epileptic patients. However, the recruited medical personnel are in the same socioeconomic status as the epileptic patients and may balance some confounding factors.

The study population, the epileptic patients treated in epilepsy clinic at Siriraj Hospital, may not represent the target population, Thai ambulatory pre-menopausal epileptic patients, because the majority of the recruited epileptic patients may live in the central part of Thailand that may not represent epileptic patients in other part of Thailand.

To set the difference of mean BMD between epileptic patients receiving AEDs 3 years or more and healthy controls lesser than 10% will require larger sample size. Regarding to scarcity of fund, large sample size is not feasible.

### 3.9.12 Expected Benefit and Application

As human lifespan is increasingly increased, osteoporosis, a major risk factor of bone fracture, could be another future great health problem particularly for post-menopausal females. Patients who need to take long-term medication with adverse effect on BMD such as AED may have the same fate. Medical attention and prevention measures for this problem should be promoted. However, prevention measures and management may have a high cost. With the result of the study, the perspective of the significance and magnitude of this problem will be more clearly documented. Further research for proper prevention measures and management can be planned in term of health economic view for efficient health resource utilization.

Since, it is established that BMD is influenced by various factors affecting bone metabolism or homeostasis of calcium and phosphorus. Vitamin D intake and vitamin D-produced by skin exposure to sunlight, calcium intake, and weight-bearing activity are among important influencing factors. It is the purpose of the study that these factors are not strictly controlled in order to be able to generalized the study result to the majority of Thai pre-menopausal female epileptic patients.