CHAPTER III

3. RESEARCH DESIGN AND METHODOLOGY

3.1 Research Questions

Can Quantitative Ultrasound (QUS) at calcaneus be used as diagnostic test in the case finding of osteoporosis in Thai postmenopausal women?

3.2 Research Objective

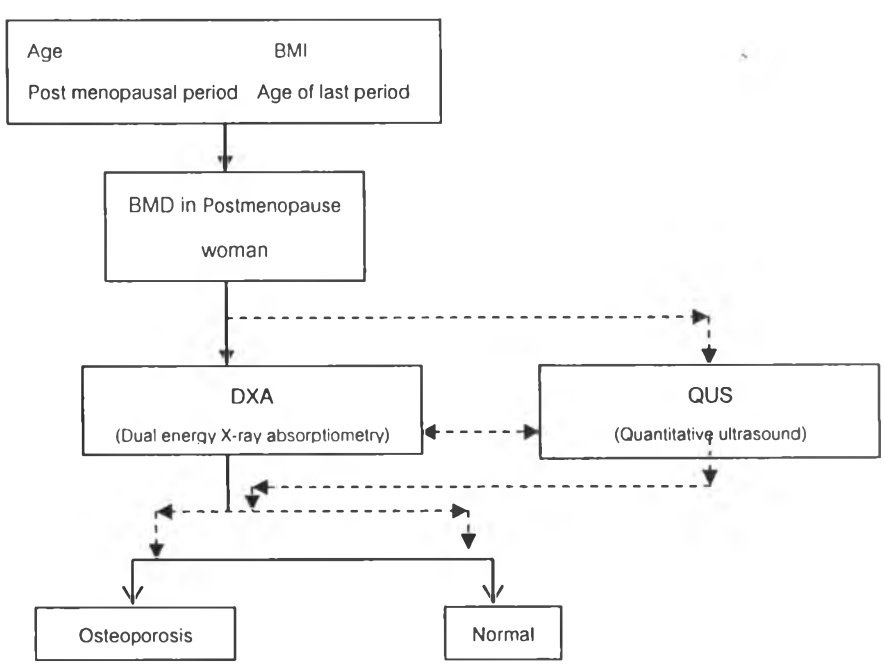
To assess the diagnostic performance of Quantitative Ultrasound (QUS) at calcaneus in detecting osteoporosis in Thai postmenopausal women using Dual energy X-ray absorptiometry (DXA) as the gold standard.

3.3 Research Hypothesis

None

3.4 Conceptual framework

Figure 3.1 Conceptual framework



Many factors influencing peak bone masses of women are age, BMI, age of last period and the duration of postmenopausal period. Recently, DXA is the gold standard

method used to determine BMD in postmenopausal women, whereas the alternative method is QUS, which the diagnostic performance will be assessed (Figure 3.1).

3.5 Assumptions :

3.5.1 DXA is the gold standard for diagnosis osteoporosis.

3.5.2 Postmenopausal women who come to out patient clinic of Nuclear Medicine Division, Phramongkutklao Hospital for BMD measurement, have menopausal conditions not different from those of other hospitals.

3.5.3 BMD was measured at left femoral neck and left calcaneous because almost patients dominated at right side so left side should be the real status of osteoporotic bone which could be determined.

3.6 Keywords

Osteoporosis, Postmenopause, Diagnosis, Dual energy X-ray absorptiometry, Quantitative ultrasound.

3.7 Operational Definitions

Menopause: The permanent cessation of menstruation resulting from the loss of ovarian follicular activity. Menopause is indicated only after 12 consecutive months of amenorrhea, regardless of pathological or physiological cause.

Postmenopause period: The period after the final menstrual period regardless of whether it was spontaneous or induced, that is duration of current age in year minus age at menopausal indicated.

Osteoporosis: A systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture[31,32]. Osteoporosis is diagnosed using The World Health Organization (WHO) criteria based on the T-score[33]of BMD determined by DXA. According to the WHO definition, subjects are classified as osteoporotic (a BMD less than the young normal mean by at least -2.5 SDs), osteopenia (a BMD between 1 and -2.5 SDs below the young normal mean) or within normal range (a BMD less than 1 SD below the young normal mean on the basis of their spine and femoral neck BMD measurements).

3.8 Research design and Research methodology

The research study is designed to reflect its objectives, as already stated, which are mainly to determine the accuracy and reliability of the QUS. The most appropriate study design is a cross-sectional descriptive study (diagnostic test). This study was carried out at the Nuclear Medicine Division, Phramongkutklao Hospital. In addition to the general study design, size and nature of sample, variables of interest, and measurement were also considered. To help making these decisions, the present knowledge regarding study design and methods in this field were searched and summarized. In the light of the limitations of previous studies (see introduction), I would like to propose a study with the following features.

3.8.1 Population and sample

3.8.1.1 Target population: Thai postmenopausal women referred by their general practitioners for bone densitometry to evaluate possible osteoporosis.

3.8.1.2 Sample population: Consecutive new postmenopausal women, who come for bone densitometry to evaluate possible osteoporosis at outpatient clinics of Nuclear Medicine Division, Phramongkutklao Hospital during June 2002 - September 2002, were recruited.

Eligible criteria

Inclusion criteria

Women who loss their normal menstruation period for at least 1 year and consent to DXA and Calcaneal Quantitative Ultrasound evaluation.

Exclusion criteria

1. Postmenopausal women with history of having old fracture of bone in the neck of femur or calcaneus.

2. Postmenopausal women who have hip or knee prosthesis.

3. Postmenopausal women who have abnormal feature of bone at calcaneus by physical examination.

4. Postmenopausal women who have history of calcification at calcaneal bone from the disease of calcaneus such as plantar fasciitis, plantar fibroma, retro calcaneal bursitis and ankle sprain/strain.

3.8.1.3 Sampling Technique: Since every subject of a given sample size in the population has equal possibility of being chosen, we may have difficultly in constructing the list and the costs involved may be prohibitive. I, therefore, decided to take a non probability sampling.

3.8.2 Sample size calculation

Sample size calculations are required to determine how many subjects should be recruited in order to meet the aims of the study. Sample size calculations require some arbitrary assumptions such as the level of statistical confidence. However, other influencing factors, which affect the sample size such as confounding factors and effect modifiers, are usually not included. Therefore, calculated sample size should not be interpreted too rigidly. To balance between the values of precision against feasibility is, instead, a reasonable way to determine how many subjects we need.

Since the consequences of missing osteoporosis cases are potentially grave, high sensitivity is preferred. It is expected that sensitivity of QUS to identify the osteoporosis by the data from Lippuner et al[18] was about 90% with 10% allowable error. In a prevalence study confined to one population, the formula for calculating sample size using 95% confidence interval[34] is :

$$n = Z^2_{\alpha/2} PQ/\delta^2$$

n = number of postmenopausal patients with osteoporosis

P = expected sensitivity of the test = 0.90, Q = 1 - P

δ = accepted error of sensitivity = 0.10

where α = type I error = 0.05 $Z_{0.025}$ = 1.96

Thus n = (1.96)(1.96)(0.90)(0.10) / (0.10)(0.10) = 35 osteoporosis patients

Due to the fact that the previous study of Lippuner et al[18] has found the sensitivity and specificity for osteoporosis of Quantitative ultrasound (QUS) at calcaneus which are 90% and 64% respectively, the sample size was therefore estimated by using sensitivity at 90%. This value was applied as a rough guideline because generalizing this value to different condition was of questionable validity.

Since the prevalence of age-adjusted prevalence of osteoporosis in Thai population was about 13.6%[28], thus, the required sample size would then be approximately n/0.136 ≈ 260 subjects.

3.8.3 Outcome measurement

3.8.3.1 Baseline variables:

Baseline data consist of both demographic and clinical variables such as age (year), height, weight, date of the last period and duration of postmenopause, and osteoporosis diagnosed by DXA.

3.8.3.2 The Main outcome:

3.8.3.2.1 Reliability: The assessment of variation due to the test method or measurement relates to the reliability of QUS. The outcomes were degree of consistency of stiffness index using the intraobserver variability, which in this case refers to test-retest differences in repeated measurements by the same screener.

3.8.3.2.2 Accuracy: Accuracy should be determined by examining representative patients with suspected condition, applying the diagnostic technology under investigation and proceeding with independent application of the gold standard. So the outcomes were sensitivity, specificity, predictive values, and likelihood ratio (95% confidence interval).

The gold standard : Diagnosis of osteoporosis by DXA instrument

The result was grams per square centimeter of DXA-Bone mass density (BMD) which was calculated by dividing the amount of bone mineral content by projected area of interest (AOI). The machine converted the bone mass density to T-scores as recommended by the WHO. In this study, the researcher did not use the value of bone mass density as a continuous variable but use a dichotomy, case versus non-case basis based on the T-score as defined by the WHO, instead. The result was therefore classified into two groups: Normal (T-score greater than -2.5 SD of the young normal mean), and Osteoporosis (T-score Less than or equal to -2.5 SD of the young normal mean).

The diagnostic technology under investigation: Diagnosis of osteoporosis by QUS instrument

BMD measured by QUS would be treated as continuous variable and also categorical variable. First, the result was expressed as Stiffness Index-Bone mass density which combines BUA and SOS value linearly, with equal weighting, into a single parameter. Stiffness index has a young adult value about 100. We used this Stiffness index as a continuous numerical variable to find the optimal cut-off point for diagnosing osteoporosis. Second, the SI was converted to T-scores as recommended by the WHO. The result was therefore classified into two groups: Normal (T-score greater than -2.5 SD of the young normal mean), and Osteoporosis (T-score Less than or equal to -2.5 SD of the young normal mean).

3.9 Data collection

Decisions on the way data should be collected depend on the nature of the information sought. This study involves data from investigations. Measurement results recorded by technologists were the principal data collected.

There were 300 consecutive new patients who are eligible for the inclusion. The general aim of the study was explained. The patients were reassured that all responses would be treated as confidential and were also informed that they were at liberty to refuse to take part of the study if they so wished. This process occurred before the patients were seen by the doctors at the outpatient clinics of Nuclear Medicine Division. A demographic data was collected by research nurse, then each subject was undergone both QUS and DXA measurements by independent technologist. The QUS was measured twice for test-retest reliability by the same technologist. The first was performed before and the second after the DXA was done. The duration of first and second measurement was not exceeded 30 minutes. We used the second time of measurement for analyzed data. The detail procedures were as follow :

Ultrasound Measurements

QUS measurement was performed with an Achilles express ultrasound device (Lunar). The Achilles express is a fully portable ultrasound system for measurement of the calcaneus using gel as a coupling agent. The two fixed transducers, is fluid-coupled, through-transmission quarter wave-matched, broadband single element (25 mm diameter) center frequency 500 KHz. During measurement, the subject puts bare left heel on the

foot-plate of the unit and a calf rest is used to aid correct positioning of the foot. Acoustic coupling between the transducers and skin was achieved with water-soluble ultrasonic gel specifically produced for this ultrasound unit. It was possible to complete each test within 3 minutes. The subject's heel was positioned on the foot support plate and strapped in place to restrain it from moving. The two transducers were positioned on either side of the approximate midpoint of the calcaneus with a constant pressure maintaining direct contact with the patient's skin. For result : we linearly combined BUA and SOS value[11,12,13], with equal weighting; into a single parameter called the Stiffness index (%). The manufacturer's reference population for stiffness index was used to calculate the T-score. Daily quality control (QC) was performed for ultrasound systems with acoustic phantoms provided by the manufacturers. After first QUS measurement, the subjects were undergone DXA investigation and would be measured QUS for the second time by the same technologist immediately.

Dual X-ray Absorptiometric Measurements

Bone mineral measurements with DXA were performed with a bone densitometer (Hologic QDR-4500 scanner; Hologic, Waltham MA) at the lumbar spine and the left proximal femur. We measured left total, neck and trochanter femoral BMD of each subject on the same day by a well-trained technologist of Nuclear medicine department. BMD (g/cm^2) was calculated by dividing the amount of bone mineral content by the projected area of the region of interest (ROI). We used the only BMD of left neck of femur. Quality assurance phantom scans were performed to check system calibration on daily basis. Technologists did not know the result of QUS measurement before doing DXA. After diagnosis of osteoporosis, the patient would be explained how to protect bone fracture in the future and how to manage osteoporosis. Then they were referred to the orthopedist or gynecologist at menopausal clinic.

3.10 Data transformation

Before analyzing the data, some variables were transformed in order to make the results convenient to interpret and be comparable with existing studies. For continuous variables, decisions had to be made on how many categories and where the category boundaries should be. There are no definite rules for categorization of continuous variables.

Age, BMI, age of the last period, and time from last period until current age were categorized using the statistical distribution such as tertiles, or quartiles as appropriate. Using this statistical distribution can spread equal numbers of observations within categories which make them become statistically more stable.

3.11 Data Analysis

3.11.1 Analysis of baseline variables: Baseline characteristics of studied population were analyzed using descriptive statistics.

The number of osteoporosis in this population was presented as percentage.

3.11.2 Association between osteoporosis and other baseline variables:

Relationship between demographic, clinical variables and osteoporosis were assessed using chi-square statistics.

3.11.3 The consistency of the test over time

The intraclass correlation coefficient was calculated basing on the degree of correspondence between the first and the second rating of QUS measurements.

3.11.4 The accuracy of the test

The diagnosis performances of QUS measurement were analyzed in two aspects. First, to determine its accuracy by assessing sensitivity, specificity, positive predictive value, and likelihood ratio. For this, both QUS-Stiffness index and DXA-BMD were used for gold standard. Second, Receiver Operator Characteristic (ROC) analysis was carried out to determine the best sensitivity and specificity for QUS by using stiffness index to compare to the gold standard and to find the best cut-off values that will give the high sensitivity.

3.12 Ethical consideration

The study protocol was thoroughly explained to the subjects before enrolling in the study. The study was approved by the Human Research Ethics Committee. The informed consent document contains a statement defining that the consent was freely given and had to be sign for every participated subject (Appendix C), the patient is aware of risks and benefits of entering the study, and the patient is free to withdraw from the study at any time.