ORIGINAL ARTICLE

Calcaneal Quantitative Ultrasound and Bone Turnover Markers for Osteoporosis Screening in Elders, the Providing of Benefits

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Abstract

Context: Calcaneal quantitative ultrasound (QUS) is attractive as a pre-screening tool for osteoporosis, alternative to dual-energy X-ray absorptiometry. Bone turnover markers (BTMs) are represented as bone formation indicators. QUS and BTM analysis may provide osteoporosis screening and bone turnover status, which provide benefit for osteoporosis management. **Aims:** The aims of this study were (1) to compare the values of bone mineral density (BMD) and BTMs, including N-terminal extension propeptide of type-I collagen, alkaline phosphatase (ALP), C-terminal telopeptide of type-I collagen (CTX), and osteocalcin (OC), and bone-relating biochemical parameters, such as 25-hydroxyvitamin D (25(OH)D), calcium, phosphorus and magnesium between osteoporotic, osteopenia, and normal BMD elders and (2) to investigate the relationship between serum BTMs and BMD. **Materials and Methods:** Determination of BMD, BTMs, and bone-related biochemical parameters from 150 of the elders at Amphawa District, Sumut Songkhram, was determined by calcaneal QUS and automatic analyzers, respectively. One-way ANOVA was used to compare continuous variables between three elder groups. Multiple comparisons among groups were used least-significance different. Pearson correlation was used to evaluate the correlation between BMD and BTMs. The statistical significance was considered at $P < 0.05$. **Results and Discussion:** BMD, calcium, phosphorus, and CTX levels were significantly different among three elder groups. CTX was significantly inversely correlated to BMD. Calcium, phosphorus, and CTX can be useful with osteoporosis screening by QUS, especially for osteopenia. **Conclusions:** Combination of calcaneal QUS and biochemical tests, including serum calcium, phosphorus, and CTX measurement as early diagnosis, provides more benefits for osteoporosis management and suitable for mass screening and intervention.

Key words: Bone mineral density, bone turnover markers, bone-related biochemical parameters, calcaneal quantitative ultrasound, osteoporosis

INTRODUCTION

Osteoporosis is the most common bone diseases, especially in elders, which defined by the reduction of bone mineral density (BMD), deterioration of bone tissue, and disruption of bone-inside structure. The reduction of bone strength in osteoporosis is a trend to increase the risk of bone fractures.[1-3] The risks of osteoporosis are increasing with age, and the highest of osteoporosis rate is at about 85 years of age. The ratios of osteoporosis diagnosis at this age are 1 in 3 women and 1 in 10 men.[4] The consequences of osteoporosis are associated by serious conditions, such as fractures, reduction and slow movement, reduced social interaction, and death.[5] In Thailand, the increment of life period is a trend to increase the number of elders and the rate of osteoporosis will be rapidly increased[6] along with common chronic diseases, such as diabetic mellitus and cardiovascular diseases.[7-9]

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The age-adjusted prevalence of osteoporosis in Thai women (40–80 years) during 2000–2001 is 13.6% and 19.8% for femoral neck and lumbar spine, respectively. In addition, the prevalence of osteoporosis in Thai men is 12.6%, 4.6%, and 3.9% at the femoral neck, lumbar spine, and both sites, respectively. Multi-national research survey for Asian osteoporosis study had reported the incidence of hip fracture in Thailand, which was 114 and 289 (per 100,000), in men and women, respectively.[6,10]

Recently, dual-energy X-ray absorptiometry (DXA) is the most common method for the evaluation of BMD to assess the risk of bone fracture, which is a reference standard for BMD measurement for diagnosis and monitoring of osteoporosis. However, it is difficult to screen small bone microarchitecture changes in a short time by BMD.[11,12] However, DXA is relatively expensive, and there is also lack of indication for examining the potential risks and benefits of undertaking the test, and ultimately, whether it is worth offering this service under the public health insurance scheme.[13] DXA for osteoporosis screening in elders is limited at rural area, which had inappropriate medical staffs and equipment. Moreover, elders at home and/or bed-bound elders are also difficult to assess and give health service. When DXA is rarely using for osteoporosis screening, the calcaneal quantitative ultrasound (QUS) is an attractive method for the screening of osteoporosis alternated to DXA. QUS is a bone assessment technique, which has gained much popularity in recent years. Comparing with DXA, QUS is more preferable on public service due to portable, simple to handle, inexpensive cost, and non-harmful method by useless ionizing radiation. The technique can be used to determine bone status in women, men, and children and in certain cases, infants.[14] The reliability of QUS for BMD assessment in diagnosis osteoporosis had been reported in Thai population.[15]

Both the intra- and inter-observer reliabilities of stiffness index of QUS in measuring BMD of the calcaneus are evaluated and concluded that QUS is the reliable tool for measuring BMD and it can be used as alternative for osteoporosis screening, particularly in areas, which are difficult to access, and limited in resources.[15] However, QUS is providing only BMD for osteoporosis diagnosis, which is unable to give information of bone turnover and/or relating biochemical bone status for the prediction of supplement effectiveness, especially in case of mass calcium and Vitamin D supplementation in community level.

Bone turnover markers (BTMs) are usually represented as bone formation indicators, including N-terminal extension propeptide of type-I collagen (P1NP), bone-specific alkaline phosphatase (BALP), and bone resorption markers, including C-terminal telopeptide of type-I collagen (CTX) and osteocalcin (OC).[16-18] They are providing information on fracture risk independent of BMD and predict the rapidity of bone loss in untreated patients, which also used to predict the response to treatments. The reduction in BTMs following antiresorptive therapy and reduction in vertebral and non-vertebral fracture risk are related, and the greater the decrease in BTM may reduce the risk of fracture. The International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine Working Group are evaluated BTMs for the prediction of fracture risk and for monitoring of treatment; they are suggested that bone formation markers (s-PINP) and bone resorption markers (s-CTX) can be used as reference markers and measured by standardized assays in observational and intervention studies.[3,16]

Thus, QUS and BTM analysis may provide BMD for osteoporotic screening and bone turnover status of bone for management rather than QUS test alone, especially in elders. Our objectives were aimed to compare the values of BMD and BTMs, including P1NP, ALP, CTX, and OC, and other relating biochemical parameter, such as 25-hydroxyvitamin D (25(OH)D), calcium, phosphorus, and magnesium between osteoporotic, osteopenia, and normal BMD elders. In addition, we were aimed to investigate the relationship between BMD and BTMs.

MATERIALS AND METHODS

Participated elders and demographic data

The cross-sectional study was carried out from November 2017 to April 2018 (this period including public relation and health service), and 150 of health customers were included from academic health service program at Amphawa district, Sumut Songkhram, which was responsible by Sumut Songkhram Education Center, Suan Sunandha Rajabhat University. Informed consent of all participants was done, and the study protocol was approved by the Ethical Review Committee from Suan Sunandha Rajabhat University. Screening of BMD was done for classified bone density status as normal, osteopenia, and osteoporotic persons.

The inclusion criteria

The following criteria were included in the study:
1. Male and female elders were aged ≥60 year
2. Consciousness and interactive
3. Had not severe medical conditions.

The exclusion criteria

The following criteria were excluded from the study:
1. Elderly was combined with metabolic bone disease (osteomalacia, Paget's disease, or primary hyperparathyroidism)
2. Had accident, especially with fracture
3. Taking daily calcium and Vitamin D supplements
4. Pathological fracture (secondary osteoporosis).[19,20]

Anthropometric data, medical history, and health behaviors (including related risks) were recorded by physical examined and interviewed. The elders with poor literacy and/or unable to read as well were helped for filling the form of questionnaire.

BMD and biochemical measurements

BMD was measured by calcaneal QUS and ultrasound bone densitometer (SONOST-2000, OsteoSys, Korea), and the instrument protocol and data interpretations were followed by manufacturer instruction. According to BMD status, elders were divided to osteoporotic (T score at or below −2.5), osteopenia (T score between −1.0 and −2.5), and normal (T score at above −1.0) elders. Each 6 ml of fasting blood sample was obtained by venipuncture from median cubital vein during the morning (7–9 a.m.). 6 ml of blood sample was drawn into clotting blood tube for centrifuged and processed within 2 h after phlebotomy and stored at −20°C.[21] Each serum concentration of BTM (P1NP, ALP, CTX, and OC), 25-(OH)D, and bone-related minerals (calcium, phosphorus, and magnesium) was determined by Cobas E411, Cobas E601, and Cobas E501 automatic analyser (Roche Diagnostics, Basel, Switzerland), respectively. The within-run and between-run CVs were <10%. Control materials and pooled serum were also done prior sample test for accuracy checking.

Statistical analysis

The Kolmogorov–Smirnov test was used to test for normal distribution. One-way ANOVA (or Kruskal–Wallis) test was used to compare the continuous variables as appropriate between three elder groups. Multiple comparisons (*post hoc* test) among groups were least-significant difference (LSD). Pearson correlation was used to evaluate the correlation between BMD and BTMs. The statistical significance was judged at $P < 0.05$. SPSS 18.0 software was used for statistical analysis (SPSS, Chicago, Illinois, USA).

RESULTS AND DISCUSSION

The elders, who participated in this study, were mainly female (88.5%) and the average age was 67 ± 5.6 years old. The average of BMD and calcium levels from all elders was lower than normal range, whereas average of CTX level was higher than normal range. Moreover, average of BMD, calcium, phosphorus, and CTX levels was significantly different among three elder groups. BMD and calcium were relatively decreased in normal, osteopenia, and osteoporosis groups, respectively; CTX level was also relative increased in normal, osteopenia, and osteoporosis groups, respectively. However, other biochemical parameters, including phosphorus, magnesium, ALP, Vitamin D (25-OH-D), OC, and total P1NP levels, were within normal ranges [Table 1]. The multiple comparisons of BMD, calcium, phosphorus, and CTX between normal, osteopenia, and osteoporosis groups were analyzed by LSD. Almost each parameter was significantly different except phosphorus level between osteopenia and osteoporosis groups which was not significantly different [Table 2]. Only CTX was one of the BTMs, which significantly negatively correlated to BMD, while OC and total P1NP were negatively and positively correlated; however, there was no statistical significance [Table 3].

Calcaneal QUS is an alternative technique for assessing bone. Compared to DXA, QUS has the advantages of being cheaper, portable, and free of ionizing radiation.[22] The systematic review on calcaneal QUS are representing usefulness as screening tool for of osteoporotic assessment. However, there is no consensus for the type of devices, measured variables, or cutoffs. Overall, there is no sufficient evidence to recommend a specific cutoff for calcaneal QUS that provides a certainty level high enough to rule in or out osteoporosis. Calcaneal QUS in a pre-screen or stratification algorithm must be based on device-specific cutoffs that are validated in the populations for which they are intended to be used.[23] However, reliable of calcaneal QUS is still used as alternative screening for diagnosing osteoporosis, acceptable of calcaneal QUS had been a report for cost-effectiveness and ease to access in rural area.[15] The calcaneal QUS for BMD assessment is able to reflect bone quality and can be used in developing countries for screening of osteoporosis, where DXA devices are less accessible to public population.[14] According to the study, T-scores of BMD measurement among three elder groups were significantly different, which was the suggestive data for calcaneal QUS for osteoporosis screening as corresponding to previous studies. In addition, the summation of osteopenia and osteoporotic elders was higher than normal, which may implied that BMD screening for osteopenia people was important due to ease to prevention by calcium supplement and no significant symptom or complication occurring.[3]

In this study, there were significantly differences of calcium, phosphorus, and CTX among three elder groups. However, other biochemical parameters, including phosphorus, magnesium, ALP, Vitamin D (25-OH-D), OC, and total P1NP levels, were within normal ranges. The finding may provide useful biochemical parameters, which were different among normal, osteopenia, and osteoporosis elder groups, and can be combined measurement with calcaneal QUS for BMD screening and for diagnosis prevention, control, or management of osteoporosis together rather than diagnosis alone. Low concentrations of serum 25-(OH)D could increase the parathyroid hormone (PTH) concentrations, resulting in higher rates of bone loss, and Vitamin D status is evaluated by measuring the serum 25(OH)D. The concentrations of serum 25-(OH)D are defined as deficient (<20 ng/ml), insufficient (20–30 ng/ml), or sufficient (≥30 ng/ml).[24-26] Thus, the monitoring of serum 25-OH-D was also necessary in case of mineral and Vitamin D supplement. However, averages of

Table 1: The difference of BMD and biochemical parameter among three groups of eldersy compared with normal range of each parameter

Parameter	Normal range	Total elders (*n*=150)	Normal BMD (*n*=65)	Osteopenia (*n*=45)	Osteoporosis (*n*=40)	*P* value
BMD	T score >−1.0	−1.70±0.89	−0.80±0.19	−1.99±0.27	−2.84±0.297	<0.001*
Calcium	8.6–10.2 mg/dL	7.66±1.38	8.87±0.54	7.30±1.00	6.09±0.78	<0.001*
Phosphorus	2.7–4.5 mg/dL	3.12±0.67	3.30±0.72	4.35±19.77	2.99±0.70	0.016*
Magnesium	1.40–2.10 mEq/L	2.10±10.82	1.70±0.029	2.09±0.50	1.45±0.28	0.362
ALP	39–105 U/L	53.88±9.30	53.45±8.44	54.67±10.62	53.70±9.22	0.789
Vitamin D (25-OH-D)	≥30 ng/mL	41.76±7.51	42.40±7.61	40.64±7.04	41.98±7.88	0.476
Osteocalcin (OC)	11.5–29 ng/mL	16.54±3.40	16.40±3.39	16.46±3.07	16.88±3.80	0.767
Total P1NP	15.0–74.0 ng/ml	46.17±15.18	46.56±15.19	46.07±15.98	45.64±14.61	0.954
Beta-CrossLaps (CTX)	0.0–0.32 ng/ml	0.38±0.15	0.30±0.10	0.40±0.12	0.50±0.15	<0.001*

BMD: Bone mineral density, *statistically significance at *P*<0.05

Table 2: Multiple comparisons of BMD, calcium, phosphorus, and beta-CrossLaps (CTX) within normal, osteopenia, and osteoporosis groups

Dependent parameter	Group	Other group	Mean difference	Standard error	95% CI		*P* value
					Lower	Upper	
BMD	G1	G2	1.196*	0.048	1.101	1.292	<0.001*
		G3	2.038*	0.050	1.940	2.137	<0.001*
	G2	G1	−1.196*	0.048	−1.292	−1.101	<0.001*
		G3	0.842*	0.054	0.735	0.948	<0.001*
	G3	G1	−2.039*	0.050	−2.137	−1.940	<0.001*
		G2	−0.0842*	0.054	−0.948	−0.735	<0.001*
Calcium	G1	G2	1.570*	0.149	1.276	1.864	<0.001*
		G3	2.778*	0.154	2.473	3.082	<0.001*
	G2	G1	−1.570*	0.149	−1.864	−1.276	<0.001*
		G3	1.208*	0.167	0.879	1.537	<0.001*
	G3	G1	−2.778*	0.154	−3.082	−2.473	<0.001*
		G2	−1.208*	0.167	−1.537	−0.878	<0.001*
Phosphorus	G1	G2	0.318*	0.128	0.066	0.570	0.014*
		G3	0.313*	0.132	0.051	0.574	0.019*
	G2	G1	−0.318*	0.128	−0.570	−0.066	0.014*
		G3	−0.005	0.143	−0.288	0.277	0.970
	G3	G1	−0.031*	0.132	−0.570	−0.051	0.019*
		G2	0.005	0.143	−0.278	0.288	0.970
Beta-CrossLaps (CTX)	G1	G2	−0.102*	0.024	−0.149	−0.055	<0.001*
		G3	−0.198*	0.025	−0.247	−0.150	<0.001*
	G2	G1	0.102*	0.024	0.055	0.149	<0.001*
		G3	−0.096*	0.027	−0.149	−0.044	<0.001*
	G3	G1	0.198*	0.024	0.150	0.247	<0.001*
		G2	0.096*	0.027	0.044	0.149	<0.001*

*Statistically significance at *P*<0.05; CI: Confident interval, G1: Normal BMD group, G2: Osteopenia group, G3: Osteoporosis group, BMD: Bone mineral density

25-OH-D among elder groups in our study were sufficient as within normal range.

OC is a non-collagenous protein regulates glucose, lipid, and energy metabolisms as well as bone metabolism.[16-18,27] P1NP is also another protein which is secreted by osteoblasts during the collagen type I synthesis. This protein has been taken into account in the osteoporosis management.[16-18,28] C-terminal cross-linked telopeptide of type-I collagen (CTX) as a bone resorptive marker is produced by osteoclasts during bone resorption.[16-18] In calcium supplement study, CTX was observed at 12 months and a greater decline in OC was observed at 1 year.[20] In our results, only CTX was significantly negatively correlated to BMD, while no statistical significant

Table 3: The correlation of bone turnover markers to BMD (*n*=150)

Bone turnover marker	BMD relation value (Pearson correlation)	*P* value
OC	−0.040	0.629
Total P1NP	0.001	0.986
Beta-CrossLaps (CTX)	−0.524**	<0.001**

**Significant at *P*<0.01 level (two-tailed). OC: Osteocalcin, BMD: Bone mineral density

of OC and total P1NP among three elder groups. Thus, CTX is BTM that can be useful with osteoporosis screening by QUS, especially in short-term calcium supplement. Osteoporosis has no clinical manifestations until there is a fracture. Moreover, osteoporosis results in a decreased quality of life increased disability-adjusted lifespan and big financial burden to health insurance systems of countries that are responsible for the care of such patients. With an early diagnosis of this disease, before fractures occur, and by assessing BMD and with early treatment, osteoporosis can be prevented.[3] Hence, we suggest that screening of osteoporosis by calcaneal QUS with measurements of bone-related biochemical parameters (including serum calcium, phosphorus, and CTX) is providing more benefit information for prevention and/or treatment of osteoporosis rather than BMD evaluation by calcaneal QUS alone. In addition, this combined measurement is suitable for mass screening and intervention.

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