Preliminary Report

Serum Concentration of Undercarboxylated Osteocalcin and the Risk of Osteoporosis in Thai Elderly Women

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The elevation of undercarboxylated osteocalcin (ucOC) means vitamin K insufficiency is significantly increased in elderly women, and is associated with high skeletal turnover, low BMD, and increased risk of osteoporotic fracture. The objective of the present study was to find out the level of ucOC and the prevalence of vitamin K deficiency in elderly Thai women.

The upper limit of normal premenopausal ucOC level was 2.314 ng/ml, represented the cut-off level for vitamin K deficiency, 39.1% of elderly women had serum ucOC concentration above this level. Women with high serum ucOC level had a significantly lower BMD of ultradistal radius, distal 1/3 of radius and 25(OH)D level, higher serum PTH and alkaline phosphatase activity than women with a normal ucOC ($p<0.05$). Serum ucOC was correlated positively with PTH ($r = 0.411$, $p<0.001$), modest negatively with serum 25(OH)D ($r = -0.17$, $p=0.013$). The ucOC level of urbanized elderly was higher than that of rural elderly.

It was concluded that vitamin K deficiency is one of the risks of osteoporosis with high prevalence in the Thai elderly especially urbanized ones, the supplement of vitamin K should be recommended in Thai osteoporotic patients especially the urbanized elderly.

Keywords: *Undercarboxlylated Osteocalcin, UOC, UcOC*

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The vitamin K cycle, established initially in relation to blood clotting, is now known to have a similar function in the transformation of bone peptides, including osteocalcin and matrix Gla-protein, converting glutamate residues in these peptides (undercarboxylated osteocalcin) into g-carboxy-glutamate (carboxylated osteocalcin)[1-3]. Many studies[4-7] showed that the undercarboxylated fraction of circulating osteocalcin (ucOC), i.e., the fraction of osteocalcin which does not bind to hydroxyapatite, is significantly increased in elderly women, suggesting an age-dependent impairment of the g-carboxylation of osteocalcin. The elevated concentrations of undercarboxylated osteocalcin implied the insufficiency of vitamin K in the elderly women, and was associated with high skeletal turnover[7], low BMD[4], and increased risk of osteoporotic fracture[6, 7]. These observations implied that vitamin K insufficiency contributed to osteoporosis development.

In Thailand, it is still unclear about the ucOC level of elderly women, there was only one study[8] about treatment of osteoporosis postmenopausal Thai women with menatetrenone-4, and it was found that menatetrenone-4 could decrease the serum level of ucOC from 10.47 ng/dl at baseline to 5.32 ng/dl after 3 months of treatment (p=0.0001) and could decrease the bone resorption marker (Beta Crosslaps) to 65.42% after 6 months of treatment (p=0.0001)
The objective of the present study was to find out the level of ucOC and the prevalence of vitamin K deficiency in elderly Thai women.

Material and Method

The present study was a part of the studies of calcidiol level of elderly women living in urban and rural areas in Khon Kaen province, Thailand[9], that

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included 234 elderly volunteers. One hundred and thirty cases lived in rural areas, and 104 in urban areas. None of the participants had paralysis or debility, a history of metabolic or hormonal disorders which might affect calcium and bone metabolism, and none had taken any medication within the last 6 months known to influence bone turnover (i.e. estrogens, selective estrogen receptor modulators, bisphosphonates, calcitonins, vitamin D, phenytoin, carbamazepine, and rifampicin). The serum parathyroid hormone samples were measured using the electrochemiluminescence (ECLIA) technique on an Elecsys 1010. The serum 25(OH)D samples were measured using the radioimmunoassay (RIA) technique by a DiaSorin, USA. The interassay coefficients of variation were 7.1 percent for the measurements of the parathyroid hormone and between 9.4 and 11.0 percent for the 25(OH)D.

The serum Glu-OC samples were assayed with enzyme immunoassay based on a sandwich method that utilizes two mouse monoclonal anti-undercarboxylated OC antibodies to detect Glu-OC by two-step procedure. One of the mouse monoclonal anti-undercarboxylated OC was immobilized onto the microtiter plate and blocked against non-specific binding. Samples and standards were added to each well and incubated. The second step was to wash the plate and to add the second anti-OC labeled with peroxidase (POD). During this incubation, Glu-OC was bound to anti-undercarboxylated OC (solid phase) on one side and tagged on the other by POD-anti OC. The reaction between POD and substrate (H_2O_2 and 3,3', 5,5' tetramethylbenzidine) resulted in color development with intensities proportional to the amount of Gla-OC present in samples and standards. The amount of Glu-OC could be quantitated by measuring the absorbance using an EIA plate reader. The intra-assay precision CV (%) = 4.58-6.06, and the inter-assay precision CV (%) = 5.67-9.87

The bone mineral density (BMD) was measured in 227 elderly women (as 7 dropped out) at Srinagarind Hospital, Khon Kaen University, using dual energy X-ray absorptiometry for 98 urban elderly who were measured by a DPX-IQ, Lunar Corp, USA, and 129 rural elderly measured by a Prodigy, Lunar Corp, USA, both machines have a precision error of 1-2%.

Statistical Analysis

Baseline demographic and clinical characteristics including age, weight, height, BMI, plasma level of undercarboxylated osteocalcin (ucOC), 25(OH)D, PTH, and alkaline phosphatase level were described as median (SE), mean (SE), minimum and maximum.

The upper limit of the normal range for ucOC that represent the cut-off level for vitamin K deficiency was calculated as the median + 2 SE in 357 healthy, Thai premenopausal women, aged 20-50 years[10]. Thus, the upper limit of normal level was 2.314 ng/ml (median = 2.100 ng/ml, SE = 0.107). If the authors used mean + 2 SE as the cut-off level for vitamin K deficiency, it was 2.91 ng/ml (mean = 2.693 ng/ml, SE = 0.107), the median rather than the mean was appropriate because the data distribution was not symmetried, it was a positively skewed distribution (skewed to the right), which caused the mean to be an overestimate, accordingly the authors decided to use "median+ 2 SE" as the upper limit of the normal range of ucOC (Fig. 1).

To compare the difference of the mean of continuous variables such as calcidiol, alkaline phosphatase, BMD and PTH level between normal vitamin K and vitamin K deficiency by unpaired t-test. Meanwhile the prevalence of vitamin K deficiency was presented as percent. For testing hypothesis, p-values<0.05 were considered statistically significant.

Results

Baseline clinical characteristics of the participants are shown in Table 1. Based on the upper limit of normal of 2.314 ng/ml for premenopausal women, 39.1% had serum ucOC concentration above the cut-off level. Women with high serum ucOC level had a significantly lower 25(OH)D level, higher serum PTH, alkaline phosphatase activity, and lower BMD of ultradistal radius

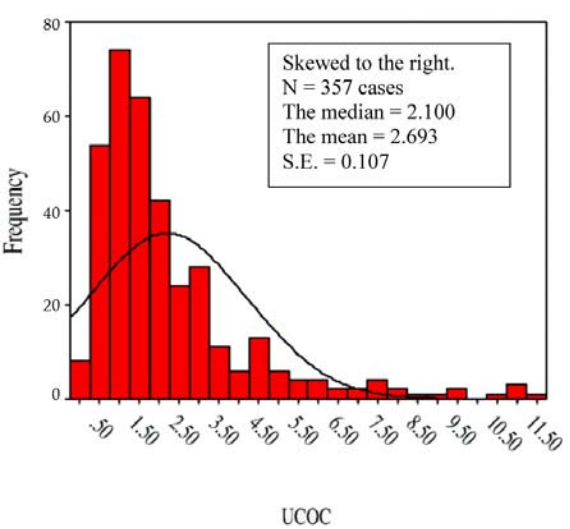

Fig. 1 The data distribution of serum ucOC of 357 cases of healthy premenopausal women, it was a positively skewed distribution (skewed to the right)

and distal 1/3 of radius than women with a normal ucOC, but the BMD of femoral neck and spine showed no significant difference (Table 2) . Serum ucOC was correlated positively with PTH (r = 0.411, p<0.001) and modest negatively with serum 25(OH)D (r = - 0.17, p=0.013). A significant positive correlation between ucOC level and PTH level persisted after excluding the effect of 25(OH)D (r = 0.38, p<0.001), but not for correlation between ucOC and 25(OH)D after excluding the effect of PTH (r = - 0.03, p=0.69)

The comparison of ucOC level between rural and urban elderly showed a significantly higher level of serum ucOC of urban elderly than rural elderly (Fig. 2)

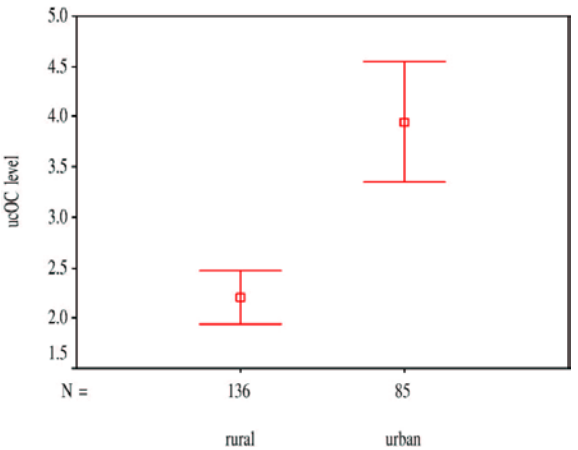

Fig. 2 Comparison of mean ± 95%CI of serum ucOC level between rural and urban elderly

Discussion

Low level of serum vitamin K causes high serum ucOC([5,7]), and is the risk for high bone turnover, low bone density and also low bone quality, that was confirmed by higher PTH and alkaline phosphatase level and lower BMD of ultradistal and distal 1/3 of radius in vitamin K deficiency group compared to normal vitamin K group. In addition, the authors also found that the urban elderly had a significantly higher ucOC level than that of the rural elderly that might be the difference in dietary habits between urban and rural elderly women.

In the present study, the prevalence of vitamin K deficiency or high ucOC level = 39.1% based on the cut-off level above 2.314 ng/ml, and caused a higher level of PTH and alkaline phosphatase. Additionally, the elderly women with high ucOC had lower bone mineral density of ultradistal radius and distal 1/3 of radius than the elderly with normal ucOC, but not for femoral neck and spine. This finding corresponded to the study of Szulc P, et al[7] and Plantalech L, et al[5], but it had a higher cut-off ucOC level than those (2.314

Table 1. The baseline characteristics of the participants

	Mean (SE)	Median (SE)	Minimum	Maximum
Age (n = 234)	70.49 (0.39)	69.50 (0.39)	60.00	92.00
Weight (n=234)	51.34 (0.72)	50.00 (0.72)	28.50	87.00
Height (n =234)	149.00 (0.37)	149.50 (0.37)	132.00	166.00
BMI (n =234)	23.06 (0.29)	23.14 (0.29)	12.67	37.17
PTH (n=234)	24.21 (1.17)	22.32 (1.17)	1.20	116.20
ucOC (n=215)	2.91 (0.16)	2.00 (0.16)	0.60	12.90
Vit D (n=234)	39.70 (0.73)	37.65 (0.73)	18.30	71.30
Alkaline phosphatase (n = 234)	88.29 (1.55)	84.00 (1.55)	24.00	192.00

Table 2. Comparison of serum biochemical characteristics and BMD values between women with normal (<2.314 ng/ml) and high (>2.314 ng/ml) ucOC level

Serum parameter and BMD	Normal ucOC mean (SE)	High ucOCMean (SE)	p-value*
25(OH)D	41.53 (1.02)	37.67 (1.13)	=0.014
PTH	19.17 (1.36)	33.50 (2.09)	<0.001
Alkaline phosphatase	84.84 (1.90)	92.71 (2.65)	=0.014
BMD ultradistal radius	0.28 (0.01)	0.26 (0.01)	=0.02
BMD distal 1/3 of radius	0.57 (0.01)	0.52 (0.01)	=0.006
BMD femoral neck	0.69 (0.01)	0.68 (0.01)	=0.522
BMD spine	0.83 (0.02)	0.81 (0.02)	=0.617

Unpaired t - test

VS 1.65 ng/ml), that might be the difference in method of measurement of ucOC, difference in racial and dietary habit, so the level of vitamin K deficiency was not of universal value, it was based on individual characteristics and method of measurement.

Conclusion

Vitamin K deficiency is one of the risks of osteoporosis with a high prevalence in Thai elderly Thai women especially urbanized ones, and the supplement of vitamin K should be recommended in Thai osteoporotic patients especially urbanized elderly women.

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ระดับของ undercarboxylated osteocalcin ในกระแสเลือดและความเสี่ยงต่อการเกิดโรคกระดูกพรุนในสตรีสูงอายุของประเทศไทย

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ในผู้สูSSอายุฎบว่ามีการเฎิ่มขึ้นขอSSระดับ undercarboxylated osteocalcin (ucOC) หรือมีการขาดวิตามินเค ในอัตราที่สูง ยังผลให้มีอัตราการหมุนเวียนของกระดูกเพิ่มขึ้น มีค่ามวลกระดูกลดลงและเพิ่มความเสี่ยงต่อการเกิดกระดูกหักจากโรคกระดูกพรุน วัตถุประสงค์ของการศึกษานี้คือต้องการหาระดับของ ucOC ในสตรีสูงอายุชาวไทย และต้องการหาความชุกของการขาดวิตามินเค

จากการศึกษาในสตรีวัยก่อนหมดประจำเดือนพบว่าค่า ucOC ที่ระดับ 2.314 ng/ml ถือเป็นระดับของการขาดวิตามินเค และพบว่าในสตรีสูงอายุที่เข้าร่วมการศึกษานี้มีความชุกของการขาดวิตามินเคสูงถึงร้อยละ 39.1 และผู้สูงอายุที่มีระดับของ ucOC สูงกว่า 2.314 ng/ml จะมีค่ามวลกระดูกของ ultradistal radius และ distal 1/3 of radius และระดับของ 25(OH)D ต่ำกว่าผู้สูงอายุที่มีระดับของ ucOC ปกติ ($p<0.05$) ผู้สูงอายุที่มีระดับ ucOC สูงกว่า 2.314 ng/ml ยังมีระดับของ PTH และระดับของ alkaline phosphatase ในน้ำเหลืองสูงกว่าผู้สูงอายุที่มีระดับ ucOC ปกติอย่างชัดเจน ($p<0.05$) นอกจากนี้ยังพบว่าระดับของ ucOC ในน้ำเหลืองมีความสัมพันธ์เชิงบวกกับระดับของ PTH ($r=0.411$, $p<0.001$) และมีความสัมพันธ์เชิงผกผันกับระดับของ 25(OH)D ($r=-0.17$, $p=0.013$) และระดับของ ucOC ในผู้สูงอายุที่อาศัยอยู่ในเขตเมืองมีระดับที่สูงกว่าผู้สูงอายุที่อาศัยอยู่ในเขตชนบทอย่างชัดเจน

จากที่กล่าวข้างต้นพอจะสรุปได้ว่าการขาดวิตามินเคถือเป็นปัจจัยเสี่ยงอย่างหนึ่งต่อการเกิด โรคกระดูกพรุน และมีความชุกที่สูงในสตรีสูงอายุ การเสริมวิตามินเคในผู้ป่วยโรคกระดูกพรุนเป็นสิ่งที่สมควรกระทำโดยเฉพาะผู้สูงอายุที่อาศัยอยู่ในเขตเมือง