Level of Undercarboxylated Osteocalcin in Hip Fracture Thai Female Patients

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The undercarboxylated osteocalcin (ucOC) level is increased in the elderly and postmenopausal women compared with the young, healthy, and reproductive women. The high level of serum UcOC reflects the vitamin K2 deficiency which not only results in high skeletal turnover, low bone quality, and low bone density, but also increases a risk of fracture. The objective of the study is to measure the ucOC level, the 25-hydroxy vitamin D (25(OH)D) levels, and the prevalence of vitamin K2 and vitamin D deficiency in Thai female patients with hip fracture.

The serum UcOC and 25(OH)D levels of the 40 female patients with hip fracture (the mean age 77.2 ± 9.7 years) were measured and compared to the control group of the 47 randomly selected female volunteers (the mean age 75.2 ± 8.1 years) without osteoporosis-related problems.

The serum level of UcOC (median) in the female patients with hip fracture was significantly higher than the control group (p = 0.0001). Fifty-three percent had the serum UcOC level above the cut-off point at 2.314 ng/ml and 83% had lower serum 25(OH)D than the cut-off point at 30 ng/ml. The serum UcOC levels; however, were not correlated with 25(OH)D levels (r = 0.191, p = 0.237).

The high prevalence of vitamin K2 and vitamin D deficiency was found in the Thai female patients with hip fracture. The additional treatment with vitamin K2 supplement besides calcium and vitamin D is a beneficial effect for osteoporosis.

Keywords: *Undercarboxylated Osteocalcin (UcOC), Hip fracture*

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Serum osteocalcin (OC), a bone specific protein containing three residues of the vitamin K-dependent gammacarboxyglutamic acid (GLA) at the position of 17th, 21st, 24th, is a widely used marker of bone formation. The synthesis of OC is vitamin D and vitamin K dependent. The precursor of osteocalcin or undercarboxylated osteocalcin (UcOC) produced by osteoblast and odontoblast are subjected to the transcriptional regulation by calcitriol; *i.e.* active form of vitamin D3. Then the undercarboxylated osteocalcin (UcOC) turns to the matured osteocalcin by vitamin K2. Thus, the level of UcOC reflects the vitamin K2 level.

The elevated concentration of UcOC implies the insufficiency of vitamin K2. The previous studies revealed that the high UcOC level was associated with high skeletal turnover[1], low bone mineral density[2], and the increase of hip fracture risks[1,3].

Previously, the UcOC level is increased significantly with age, especially in elderly women[4]. In one cross-sectional study, the vitamin K2 level was significantly lower in the elderly women with hip fracture than in the age-matched healthy controls[5]. Neverthless, the UcOC level is really affected by race, place of living, and dietary habit.

In Thailand, there were few studies about the UcOC level in the reproductive and elderly Thai

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females. Moreover, the UcOC level of hip fracture patients has never been reported. The aim of this study is to measure the UcOC level, the 25-hydroxy vitamin D (25(OH)D) level, and the prevalence of vitamin K2 and vitamin D deficiency in Thai female patients with hip fracture.

Material and Method

Material

Forty female patients (the mean age 77.2 $\pm$ 9.7 years) and 19 male patients (the mean age 75.1 $\pm$ 8.2 years) with hip fracture were included. The information of the male group was collected because no research had done on this gender. It could be a reference sometime. The study was done at Thammasat Hospital during October 2007-October 2008. None of the participants had paralysis or debility, renal insufficiency, or history of metabolic or hormonal disorders which might affect calcium or other metabolism. None has taken any medications acceptably influencing the bone turnover *e.g.* estrogens, selective estrogen receptor modulators, bisphosphonates, calcitonins, vitamin D, phenytoin, carbamazepine, rifampicin, and warfarin within 6 months prior to the study.

The control group of 47 female volunteers (the mean age 75.2 + 8.1 years) who came to the orthopedic outpatient clinic with other problems and had no evidence of osteoporosis were randomly selected by using the same exclusion criteria as those with hip fractures.

Method

The serum levels of calcium, creatinine, albumin, and alkaline phosphatase were measured by standard laboratory methods. The serum 25-hydroxyvitamin D (25(OH)D) was measured by using Electrochemilu-minescence's immunoassay (ECLIA) technique designed by Elecsys (Roche Diagnostics GmbH, Mannheim, USA) which collected 3 ml of blood in EDTA-anticoagulated tubes.

The serum UcOC level was measured by using the enzyme immunoassay, Glu-OC EIA kit®, Takara. These assay tests for monoclonal antibody specifically targeted UcOC by utilizing a novel set of monoclonal antibodies which was highly reactive to the decarboxylated osteocalcin and less reactive to carboxylated osteocalcin at position 17,21,24.

The blood collected for UcOC and vitamin D assays was centrifuged at 3000 rpm for 15 minutes. All serum samples were stored at -30°C until they were assayed.

Statistical analysis

The baseline demographic and clinical characteristics: age, plasma levels of calcium, creatinine, alkaline phosphatase, 25(OH)D, and UcOC were described as the mean, median, standard deviation (SD), standard error (SE), and 95% confidence interval (95% CI).

The upper limit of the normal range for UcOC that represents the cut-off point for vitamin K2 deficiency was calculated by using median + 2SE from 357 healthy Thai premenopausal women, aged 20-50 that equals 2.314 ng/ml[6,7] (median = 2.100 ng/ml, SE = 0.107). The median was used as a consequence of the data distribution. The distribution was not symmetrical, positively skewed (skewed to the right), and caused the mean to be overestimated (Fig. 1).

The vitamin D level at 30 ng/ml was considered to be the cut-off point for the vitamin D adequacy in order to maintain the parathyroid hormone (PTH) level within a stable range. When the vitamin D level was lower than 30 ng/ml, it was deficient[8-11].

The baseline clinical characteristics of the study group female patients with hip fracture: age and the levels of creatinine, calcium, and alkaline phosphatase were compared to those without fracture by using the unpaired student's t-test. The UcOC levels were compared by using Mann Whitney-U-test. The prevalence of vitamin K2 and vitamin D deficiency were presented in percent. The correlation between UcOC and 25(OH)D was shown by scatter diagram

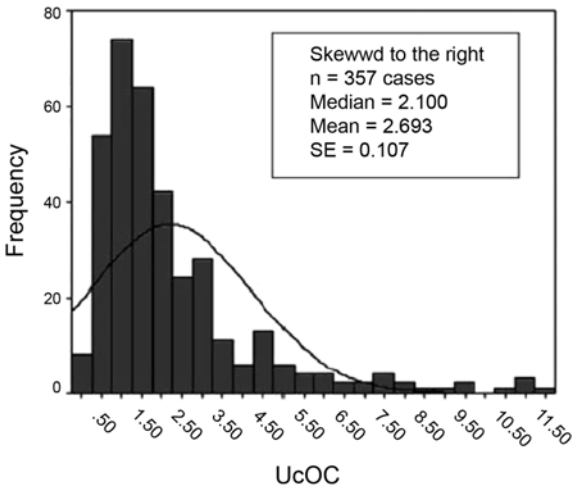

Fig. 1 The data distribution of the serum UcOC of 357 healthy premenopausal women[6]. It was a positively skewed distribution (skewed to the right)

and Pearson product-moment correlation coefficient. Testing the hypothesis was $p < 0.05$.

This study was approved by the Local Ethical Committee (MTU-P-1-039/50), and all the participants presented the written informed.

Results

The baseline characteristics of the elderly controls and the female patients with hip fracture were shown in Table 1. There were no significant differences of age and the plasma calcium, creatinine, alkaline phosphatase levels between these 2 groups.

The serum UcOC levels were significantly increased in the hip fracture patients compared to the control group ($p = 0.0001$). The serum UcOC levels of both groups were shown in Fig. 2 and 3, respectively.

Based on the upper limit of UcOC level at 2.314 ng/ml and the cut-off point for vitamin D level at 30 ng/ml, 53% of the patients had the UcOC level above this point, and 83% of the patients had the vitamin D level lower than the cut-off point. The 25(OH)D level of this group of patients is shown in Fig. 4. According to Pearson product-moment correlation coefficient, the serum UcOC was not correlated significantly with the 25(OH)D ($r = 0.191$, $p = 0.237$).

The data of the male patients with hip fracture were shown in Table 2. Sixteen percent of the patients had the higher UcOC level than the cut-off point. Sixty-three percent of the patients had the serum level of 25(OH)D which was lower than 30 ng/ml. As there was no study on male subjects, this data could not be compared.

Discussion

The high level of serum UcOC reflected the low level of serum vitamin K2[1,4] which could lead to high bone turnover, low bone density, low bone quality, and increasing risks of fragility fracture. In the present study, there was a significant increase of the serum UcOC level in the female patients with hip fracture compared to the elderly controls. The prevalence of vitamin K2 deficiency, high UcOC level, based on the cut-off level above 2.314 ng/ml, was 53% which was higher than the previous finding that was only 39.1% of the elderly women in Khonkaen, Thailand, had vitamin K2 deficiency[7]. The higher prevalence in this study resulted from the osteoporosis establishment in all patients, in contrast to the normal, osteopenia or osteoporotic population in the previous research.

Vitamin D is one of another major lipophilic vitamin. The vitamin D deficiency is common in the

Table 1. The baseline clinical characteristics of the hip fracture female patients and the elderly control

	Control n = 47	Hip fr. (female)
Age (year)	75.1 ± 8.2	77.2 ± 9.7
Calcium (mg/dl)	8.65 ± 0.62	8.59 ± 0.78
Creatinine (mg/dl)	0.73 ± 0.21	0.83 ± 0.26
Alkaline phos. (IU/L)	101.1 ± 30.5	103.55 ± 39.36

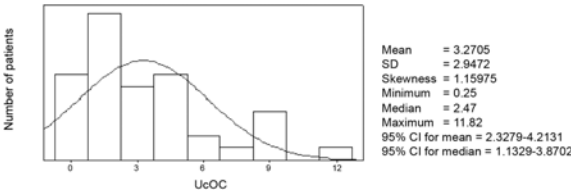

Fig. 2 The data distribution of the UcOC level of the Thai female patients with hip fracture (n = 40)

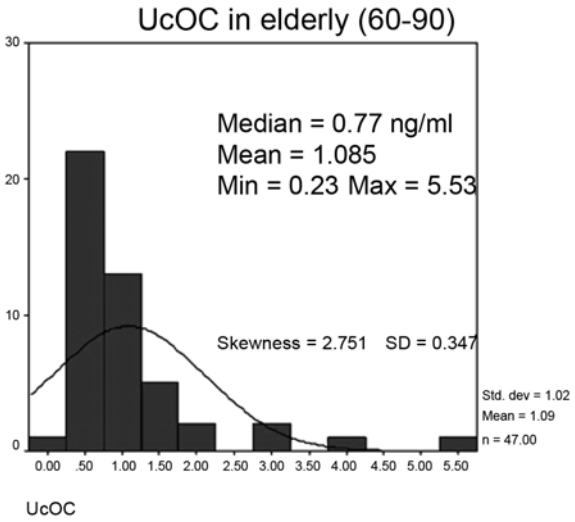

Fig. 3 The data distribution of the UcOC level of the Thai elderly females (n = 47)

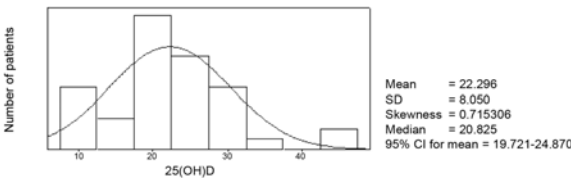

Fig. 4 The data distribution of the 25(OH) D levels of the Thai female patients with hip fracture (n = 40)

Table 2. The baseline characteristics, the serum UcOC, and the vitamin D level of male patients with hip fracture

	Mean ± SD	Median	95% CI for mean	95% CI for median
Age (year)	75 ± 8	75		
Calcium (mg/dl)	8.27 ± 0.52	8.4		
Creatinine (mg/dl	1.13 ± 0.36	1.1		
Alkaline phosphatase (IU/L)	125.47 ± 1.70	107		
UcOC (ng/ml)	1.58 ± 1.70	0.84	0.76-2.4	0.5-1.52
25(OH) D3 (ng/ml)	29.08 ± 8.29	27.44	25.08-33.08	23.35-36.52

elderly[12] due to low intake, and reduction in sunlight exposure. Eighty-three percent of the study population had the vitamin D deficiency. The treatment with 800 IU of vitamin D and calcium can prevent bone loss in the postmenopausal women and substantially reduce the risks of osteoporotic fracture[13].

The vitamin K2 supplement (Menatetrenone, 45mg/day) can decrease the serum level of UcOC to 51.52% and 87.26% at 6th and 12th months respectively. Vitamin K2 can also decrease the bone resorption marker (Betacrosslab) to 65.42% within 6 months[14].

In summary, the Thai female patients with hip fracture had high prevalence of vitamin K2 and D deficiency (53%, 83% respectively). The treatment of osteoporosis and prevention of osteoporotic fracture in the future by using vitamin K2 supplement besides calcium and vitamin D, can also be additional treatment for Thai female patients with hip fracture. Sixteen per-cent and sixty-three percent of male patients with hip fracture had the vitamin K2 and vitamin D deficiency, respectively. According to no of data base line, the results of the male patients cannot be further discussed. However, this collected information can be fundamentally used in the future although the sample size was too small (n = 19).

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การศึกษาระดับ undercarboxylated osteocalcin ในผู้ป่วยหญิงไทยกระดูกสะโพกหัก

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จากการศึกษาในสตรีสูงอายุวัยหมด ประจำเดือน พบว่าระดับ undercarboxylated osteocalcin (UcOC) สูงขึ้นเมื่อเทียบกับระดับ UcOC ในสตรีวัยเจริญพันธุ์ การที่ระดับ UcOC มีค่าสูงผิดปกติ บ่งบอกถึงภาวะการขาดวิตามินเคสองในกระดูก ซึ่งจะส่งผลให้มีการเพิ่มอัตราการหมุนเวียนของกระดูก คุณภาพของกระดูกลดลง ค่ามวลกระดูกลดลง และเพิ่มความเสี่ยงต่อการเกิดกระดูกหักจากโรคกระดูกพรุน วัตถุประสงค์ของการศึกษานี้คือต้องการหาระดับของ UcOC และ 25-hydroxyvitamin D (25(OH)D)ในผู้ป่วยหญิงไทยกระดูกสะโพกหัก และความชุกของการขาดวิตามินเคสองและวิตามินดี

การศึกษานี้ตรวจหาระดับ UcOC และ 25(OH)D ในผู้ป่วยหญิงไทยกระดูกสะโพกหักจำนวน 40 ราย (อายุเฉลี่ย 77.2 $\pm$ 9.7 ปี) เปรียบเทียบกับกลุ่มตัวอย่างซึ่งเป็นสตรีไทยสูงอายุ 47 ราย (อายุเฉลี่ย 75.2 $\pm$ 8.1 ปี) ผลการศึกษาพบว่าในผู้ป่วยหญิงไทยกระดูกสะโพกหัก มีระดับ UcOC สูงกว่ากลุ่มตัวอย่าง อย่างมีนัยสำคัญทางสถิติ ($p = 0.0001$) นอกจากนี้ยังพบว่าร้อยละ 53 และ 83 ของผู้ป่วยหญิงไทยกระดูกสะโพกหักมีความชุกของการขาดวิตามินเคสอง และความชุกของการขาดวิตามินดีตามลำดับ โดยที่ระดับ UcOC ไม่สัมพันธ์กับระดับ 25(OH)D ($r = 0.191$, $p = 0.237$) เนื่องจากผู้ป่วยหญิงไทยกระดูกสะโพกหักมีความชุกของการขาดวิตามินเคสองและวิตามินดีสูง ดังนั้นการให้วิตามินเคสองเพื่อรักษาภาวะกระดูกพรุนในผู้ป่วยกลุ่มนี้ร่วมกับการให้แคลเซียมและวิตามินดีก็เป็นอีกทางเลือกหนึ่งที่น่าสนใจ