

The Intriguing Correlation between Undercarboxylated Osteocalcin and Vitamin D

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Osteocalcin (OC) or bone Gla protein (BGP), a bone specific protein, is one of circulating biomarkers that can be used to determine the quality of bone tissues. It is synthesized by the osteoblast and the odontocyte. The process of OC production requires two types of vitamin: vitamin D and vitamin K. Vitamin D is required for the production of immature OC or undercarboxylated OC (UcOC) whereas vitamin K is required for the transformation of UcOC to mature OC. In the past, several researchers believed that UcOC was the sensitive marker to determine vitamin K status. In fact, the level of UcOC also depends on vitamin D. This study aims to know the correlation between the levels of vitamin D and UcOC. The collected data of UcOC and vitamin D levels from 95 healthy volunteers were analyzed. The finding was that there was a weak correlation between UcOC and vitamin D ($r = 0.238$; $p = 0.020$) together with UcOC and reciprocal of vitamin D ($r = 0.267$; $p = 0.009$). From this correlation, the serum level of UcOC may be not the real marker for vitamin K.

Keywords: Undercarboxylated osteocalcin, Vitamin D, Vitamin K, Total vitamin D, 25 (OH) D

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Vitamin D is a circulating prohormone. It can be synthesized in the skin upon exposure to sunlight. The active form of vitamin D requires two sequential hydroxylation steps: first, in the liver, to 25-hydroxyvitamin D (25 (OH) D₃ and 25 (OH) D₂), and second in the kidney, to 1 α , 25-dihydroxyvitamin D₃ (1 α , 25 (OH) 2D₃ and D₂)⁽¹⁾. It plays many important roles in the body such as control calcium homeostasis, regulates bone resorption as well as formation etc^(1,2).

Vitamin K is fat-soluble vitamin. It is necessary for post-translational modification of certain proteins such as osteocalcin (OC) and coagulation factors. The carboxylation of undercarboxylated osteocalcin (UcOC) needs vitamin K as a cofactor. After carboxylation, OC can function in mineral deposition⁽³⁻⁹⁾.

Osteocalcin (OC) or bone Gla protein (BGP) is a bone specific protein. It consists of 49 amino acids

synthesized by the osteoblast and the odontocyte. The development of osteocalcin needs two steps for formation: 1) the production of immature osteocalcin or UcOC, which needs an active form of vitamin D to be a cofactor, 2) the transformation of UcOC to be matured osteocalcin by vitamin K₂⁽¹⁰⁻¹³⁾.

Many researchers are interested in the correlation of vitamin D and OC. The positive correlation between vitamin D and OC were found in the studies by Bunyaratavej et al, Buranasinsup et al^(11,14) and Torbergsen et al⁽¹⁵⁾. The findings of the former research⁽¹¹⁾ were that vitamin D at concentration below 30 ng/ml had correlation with UcOC, $r = 0.032$, whereas vitamin D at concentration 30-41 ng/ml had the correlation with UcOC, $r = 0.274$. In addition, correlation of vitamin D and UcOC was also found to occur only in males, not females, $r = 0.39$ ⁽¹⁴⁾. Torbergsen et al⁽¹⁵⁾ found the correlation of 25 (OH) D and total OC with Spearman's $\rho = 0.20$. However, some researchers, for instance, Szulc et al⁽¹⁶⁾ and Saadi et al⁽¹⁷⁾ found a negative correlation between UcOC and vitamin D. Szulc et al reported that correlation of UcOC and vit D, $r = -0.24$, while Saadi et al reported that $r = -0.13$. Thus, correlation between vitamin D and UcOC are ambiguous.

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Material and Method

Subjects

This research studied 95 healthy volunteers (males = 18, females = 77), aged between 23-89 years old. No volunteers took any kind of medicine affecting bone metabolism within one month before being screened. At the screening visit, the overnight fasting blood samples were collected and examined for liver function, renal function and sugar and cholesterol levels. The subjects, who had an abnormal level of the chemicals in laboratory tests, were excluded. Vitamin D was examined at the screening visit, whereas UcOC, analyzed serums, were stored at -80°C until they were assayed.

Vitamin D and UcOC analysis

The vitamin D test as a total D, 25 (OH) D or 25 (OH)/D3D2 and 25 (OH) D was performed by Elecsys Cobas Analysis, Roche. The blood samples were analyzed by the central laboratory of Siriraj Hospital.

Serum UcOC (Takara, Japan) was analyzed by the enzyme-linked immunosorbent assay (ELISA) according to the protocol of manufacturer.

For the UcOC analysis, 100 ml of serum and the standard solution were added into the 96-well plate and incubated for 2 hours. After removing the solution and performing the wash, 100 ml of anti-UcOC conjugated with horseradish peroxidase was plated into a 96-well plate and incubated for 1 hour. The substrate solution was added to develop color. The reaction was stopped by adding 100 ml of a stop solution and the optical density was measured at 450 nm. The concentration of UcOC was shown in ng/ml.

Statistical analysis

The statistical analysis was performed by SPSS version 19 to show the mean, standard deviation and linear correlation (r) between UcOC and 25 (OH) D.

Results

Vitamin D and UcOC levels are shown in Table 1 as the mean and standard deviation.

There were the correlation between UcOC and vitamin D or 25 (OH) D, $r = 0.238$ ($p = 0.020$) as shown in Fig. 1 together with the correlation between UcOC and reciprocal of vitamin D, $r = 0.267$ ($p = 0.009$) as shown in Fig. 2.

Discussion

Several investigators^(9,18-21) reported that

Table 1. Vitamin D and UcOC

| Markers | Mean \pm SD |
|---------------------------------------------|------------------|
| Undercarboxylated osteocalcin (UcOC), ng/ml | 0.89 \pm 0.56 |
| Vitamin D, ng/ml | 46.98 \pm 19.5 |

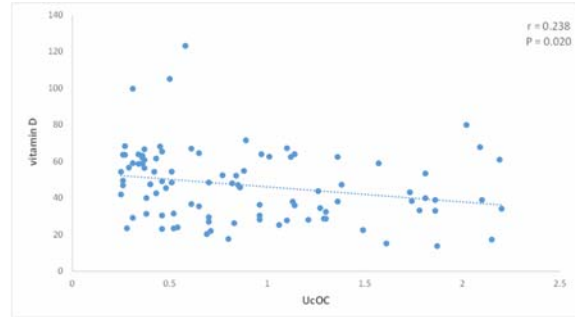


Fig. 1 The correlation (r) between the UcOC and vitamin D or total 25 (OH) D showed partial correlation ($r = 0.238$, $p = 0.020$).

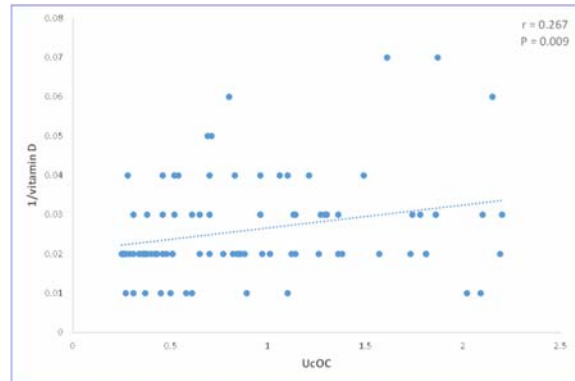


Fig. 2 The correlation (r) between the UcOC and reciprocal of vitamin D showed partial correlation ($r = 0.267$, $p = 0.009$).

UcOC is a sensitive marker for vitamin K status. Vitamin K can reduce the serum UcOC level^(3,4,6,7,18,19,22-27), and increase the serum carboxylated OC (Gla-OC)^(28,29). However, the synthesis of UcOC is vitamin D dependent⁽⁴⁾. This was confirmed by the previous experiments⁽³⁰⁾. The finding (of the previous study) was that the addition of vitamin D and vitamin K into osteogenic induction medium could increase the expression of UcOC from human periosteum derived cells (hPDCs) more than osteogenic medium without vitamin D and vitamin K⁽³⁰⁾. The other researchers Hu et al⁽³¹⁾ and Kato et al support the increasing production

of osteocalcin by vitamin D⁽³²⁾. In addition, from this study, we also found the correlation of UcOC and vitamin D together with correlation of UcOC as a reciprocal of vitamin D. Thus, it is possible that the synthesized of UcOC will not occur when the level of vitamin D is not appropriate. In this situation, the level of UcOC may not be the real marker for vitamin K. This explains why the correlation between UcOC and vitamin D is weak. We thought that since analyzed vitamin D was 25 (OH) D, not the active form 1, 25 (OH) D, the vitamin D used to synthesize UcOC was 1, 25 (OH) D. This concept comes from van der Meijden et al who found that 25 (OH) D3 can affect osteoblast differentiation via conversion to active metabolite 1, 25 (OH) 2D3 and 24R, 25 (OH) 2D3, whereas the action of 25 (OH) D3 to osteoblast function still needs further investigation⁽³³⁾. From all of the reasons explained above, it can be concluded that the serum level of UcOC may not be the real marker for vitamin K.

Potential conflicts of interest

None.

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เรื่องนํารูระหว่างอัลเดอโรออสติโอแคลซินและวิตามินดี

ชุตีเพ็ญ บุรณะสินทรัพย์, ณรงค์ บุญยะรัตเวช

ออสติโอแคลซินเป็นโปรตีนที่จำเพาะต่อกระดูกชนิดหนึ่ง และจัดเป็นโบนมาร์เกอร์ในกระแสเลือดที่สามารถใช้ในการประเมินคุณภาพของ กระดูก ออสติโอแคลซินถูกสังเคราะห์จากออสติโอบาสและออสติโอซัยต์ในขบวนการสังเคราะห์ออสติโอแคลซินจำเป็นต้องมีวิตามินที่สำคัญ 2 ชนิด คือ วิตามินดี และวิตามินเค วิตามินดี มีความสำคัญต่อขบวนการสร้างออสติโอแคลซินชนิดยังไม่สมบูรณ์ (อัลเดอโรออสติโอแคลซิน, ยูซีโอซี) ในขณะที่วิตามินเค มีบทบาทสำคัญในการเปลี่ยนออสติโอแคลซินชนิดไม่สมบูรณ์ให้กลายเป็นออสติโอแคลซินที่สมบูรณ์ในอดีต นักวิจัยมีความเชื่อว่าอัลเดอโรออสติโอแคลซิน เป็นมาร์เกอร์ที่บ่งถึงระดับของวิตามินเคในร่างกาย อย่างไรก็ตามเนื่องจากการสังเคราะห์อัลเดอโรออสติโอแคลซินขึ้นอยู่กับระดับของวิตามินดีด้วย ดังนั้นงานวิจัยนี้จึงต้องการทราบความสัมพันธ์ระหว่างระดับของวิตามินดีและอัลเดอโรออสติโอแคลซิน ผู้นิพนธ์ได้ทำการวิเคราะห์ระดับของอัลเดอโรออสติโอแคลซิน และวิตามินดีจากอาสาสมัครที่มีสุขภาพดีจำนวน 95 คน พบว่าระดับของอัลเดอโรออสติโอแคลซินและวิตามินดี มีความสัมพันธ์กันโดยมีค่าสหสัมพันธ์ (r) เท่ากับ 0.238 ที่ระดับความเชื่อมั่น (p) เท่ากับ 0.020 ในขณะเดียวกัน อัลเดอโรออสติโอแคลซินก็มีความสัมพันธ์กับส่วนกลับของวิตามินดีด้วย โดยมีค่าสหสัมพันธ์ (r) เท่ากับ 0.267 ที่ระดับความเชื่อมั่น (p) เท่ากับ 0.009 จากความสัมพันธ์ดังกล่าวนี้ จึงไม่สามารถบ่งชี้ได้อย่างเต็มที่ว่าระดับของอัลเดอโรออสติโอแคลซินเป็นมาร์เกอร์ที่บ่งถึงระดับของวิตามินเคในร่างกาย
