Special Article

Correlation between the Circulating Bone Markers and Vitamin D in the Healthy Thai People

Shutipen Buranasinsup PhD*,
Arunee Jangsangthong BS*, Narong Bunyaratavej MD**

** Department of Pre-clinic and Applied Animal Science, Faculty of Veterinary Science, Mahidol University, Nakornpathom, Thailand*
*** Department of Orthopaedic Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

There are several circulating bone markers that are useful for physicians in determining the bone quality. The markers are composed of N-terminal propeptide of type I collagen (P1NP) which determines the bone formation, undercarboxylated osteocalcin (UcOC) and N-mid fragment of osteocalcin (N-MID) which determines the bone remodeling, C-telopeptide of type I collagen (βCTx) or betacrosslap (βCTx), pyridinoline (PYD) and deoxypyridinoline (DPD) which determines the bone resorption. The vitamin named vitamin D (Vit D) also affects the bone property. However, there is little information about the relationship of these biomarkers. In this experiment, the researchers investigated the correlation of the circulating biomarkers and found the correlations between UcOC and βCTx (r = 0.471, p = 0.011), between UcOC and Vit D (r = 0.39, p = 0.04), between N-MID and P1NP (r = 0.833, p = 0.000), between N-MID and βCTx (r = 0.641, p = 0.000) and lastly between P1NP and βCTx (r = 0.657, p = 0.000) in the male group whereas the correlations between UcOC and PYD (r = 0.318, p = 0.043), between UcOC and DPD (r = 0.551, p = 0.008), between N-MID and P1NP (r = 0.721, p = 0.000), between N-MID and βCTx (r = 0.719, p = 0.000), between N-MID and PYD (r = 0.485, p = 0.001), between N-MID and Vit D (r = 0.347, p = 0.026), between P1NP and βCTx (r = 0.632, p = 0.000), between P1NP and PYD (r = 0.312, p = 0.047), between bCTx and PYD (r = 0.365, p = 0.019), between PYD and DPD (r = 0.567, p = 0.006) and lastly between PYD and Vit D (r = 0.409, p = 0.008) were found in the females. In addition, the new biomolecule named matrix Gla protein (MGP), a small protein produced by bone tissues was also investigated. The authors found the correlation between MGP and PYD (r = 0.468, p = 0.012) in the males and found the correlations between UcOC and MGP (r = 0.421, p = 0.006), between N-MID and MGP (r = 0.333, p = 0.033), between MGP and PYD (r = 0.471, p = 0.002), between MGP and DPD (r = 0.472, p = 0.026) in the female group.

Keywords: *Correlation of bone biomarkers, Vitamin D, Matrix Gla protein, Betacrosslap (βCTx)*

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There are several circulating biomarkers that can be used to determine the quality of bone tissues. For example, N-terminal propeptide of type I collagen (P1NP) is used to foretell the bone formation whereas undercarboxylated osteocalcin (UcOC) and N-mid fragment of osteocalcin (N-MID) are used to forecast the bone remodeling. Telopeptide of type I collagen (βCTx), pyridinoline (PYD) and deoxypyridinoline (DPD) are used to predict the bone resorption[1-9]. The vitamin, *i.e.* vitamin D (Vit D) can also affect the bone quality[10-13]. Moreover, the new biomolecule, matrix Gla protein (MGP), a small protein produced by bone is examined[14]. However, the information about the relationship among these circulating biomarkers is very little available. In this experiment, the researchers aim to investigate the correlations between these biomarkers.

Correspondence to:
Bunyaratavej N, Department of Orthopaedic Surgery, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.
Phone: 0-2272-0993
E-mail: todrnarong@yahoo.com

Material and Method

Subjects

This research studied 69 healthy volunteers (males = 28, females = 41), aged between 20-50 years old. All volunteers did not take any kinds of medicine affecting the bone metabolism within one month before being screened. At the screening visit, the overnight fasting blood samples were collected and examined for the liver function, the renal function as well as the level

of sugar and cholesterol. The subjects who had an abnormal level of the chemical laboratory test were excluded. Some bone biomarkers including βCTx, P1NP, N-MID, and the relevant biomolecule called Vit D were examined in the screening visit. The other bone markers, *i.e.* UcOC, MGP, PYD and DPD were also collected; the analyzed serums were stored at -80°C until they were assayed.

Markers of bone turnover

Serum UcOC (Takara, Japan), serum MGP (Biomedica, German), serum PYD (Quidel, USA) and serum DPD (Quidel, USA) were analyzed by the enzyme-linked immunosorbent assay according to the protocol of manufacturer.

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

For the MGP analysis, 20 µl of serum, the control and the standard solutions were added into the 96-well plate followed by 200 µl of biotinylated synthetic MGP (tracer) and incubated at 4°C for overnight. After removing the solution, 200 µl of streptavidin conjugated with horse radish peroxidase was added. After the incubation at RT for 1 hour, the conjugated solution was removed. The TMB substrate solution in volume of 200 µl was plated and incubated at RT for 30 minutes. Finally, 50 µl of stop solution was added and the optical density was measured at 450 nm and at 620 nm. The concentration of MGP was shown in nMol/L

For the serum PYD analysis, approximate 200 µl of serum was filtrated through MWCO Spinfilter. Twenty-five microliters of filtrated serum, the standard, low and high control solutions were added into the 96-well plate followed by 75 µl of cold anti-PYD conjugated with the alkaline phosphatase. This step was incubated at 2-8°C for 24 hours. After the incubation period, all solutions were removed from the 96-well plate. One hundred and fifty micro liters of substrate was added into the well plate and incubated for 40 minutes. Finally, 100 µl of the stop solution was added and the optical density was measured at 405 nm. The concentration of PYD was shown in nMol/L and transformed into ng/ml later.

For the serum DPD analysis, the analyzed procedure was divided into 2 steps: sample preparation and analysis procedure. For the sample preparation, 100 µl of serum was hydrolyzed by mixing with 100 µl of total DPD acid and centrifuged at 10,000 g for 5 minutes. After that, the upper solution was transferred to the hydrolysis plate and incubated at 99°C for 20 hours. Thy hydrolyzed serum was neutralized by 25 µl of total DPD assay buffer and 25 µl of total DPD base. The standard and control solutions were also neutralized by the total DPD buffer and the DPD base. The analysis procedure was started by adding 50 µl of the neutralized, standard and control serums into the 96-well plate. This step was incubated for 30 minutes at 2-8°C. The anti-DPD conjugated with the alkaline phosphatase was plated and incubated for 2 hours at 2-8°C. After removing the solution and doing the wash, 150 µl of the substrate solution was added and incubated for 2 hours at RT. Finally, 100 µl of the stop solution was added and the optical density was measured at 405 nm. The concentration of DPD was shown in nMol/L and transformed into ng/ml later.

In addition, the vitamin D test as 25 (OH) D3 was performed by Elecsys Cobas e analysis, Roche. The blood samples were analyzed by the central laboratory of Siriraj hospital.

Results

All analyzed circulating bone biomarkers are shown in the mean $\pm$ SD separated by the males group in Table 1 and the females in Table 2. On the other hand, the bone marker correlations of the males group and the females are shown in Table 3 and Table 4, respectively.

Discussion

The circulating biomolecules are useful for physicians to determine the bone quality of patients. Many researchers[15-19] have studied the correlation between biomolecules and bone mineral density (BMD). However, the availability of the information about the correlation with biomolecules is quite rare. This experiment found the positive correlations between N-MID osteocalcin and P1NP, between N-MID osteocalcin and βCTx (r = 0.719), between MGP and PYD and lastly between P1NP and βCTx in both male and female groups (Table 3 and Table 4) the N-MID osteocalcin and βCTx in the previous study[20] was strong correlation, r = 0.776 so for safe the expense. The βCTx can be presented only for the bone resorption marker. On the other hand, it found the

Table 1. Biochemical Bone Markers in the Males

Markers	The Mean ± SD	n
Undercarxylated osteocalcin (UcOC), ng/ml	2.17 ± 1.53	28
N-mid fragment of osteocalcin (N-MID), ng/ml	25.53 ± 7.77	28
Matrix Gla protein (MPG), nMol/L	24.51 ± 9.89	28
N-terminal propeptide of type I collagen (P1NP), ng/ml	53.50 ± 18.8	28
Telopeptide of type I collagen (βCTx), ng/ml	0.50 ± 0.16	28
Pyridinoline (PYD), ng/ml	0.50 ± 0.09	28
Deoxypyridinoline (DPD), ng/ml	3.72 ± 0.42	17
Vitamin D, ng/ml	20.86 ± 4.26	28

Table 2. Biochemical Bone Markers in the Females

Markers	The Mean ± SD	n
Undercarxylated osteocalcin (UcOC), ng/ml	1.36 ± 0.72	41
N-mid fragment of osteocalcin (N-MID), ng/ml	19.32 ± 5.92	41
Matrix Gla protein (MGP), nMol/L	17.59 ± 7	41
N-terminal propeptide of type I collagen (P1NP), ng/ml	38.78 ± 14.12	41
Telopeptide of type I collagen (βCTx), ng/ml	0.30 ± 0.14	41
Pyridinoline (PYD), ng/ml	0.78 ± 0.18	41
Deoxypyridinoline (DPD), ng/ml	3.49 ± 0.32	22
Vitamin D, ng/ml	18.56 ± 4.68	41

correlation between UcOC and βCTx ($r = 0.471$, $p = 0.011$) only in the males whereas the correlations between UcOC and PYD ($r = 0318$, $p = 0.043$), between UcOC and DPD ($r = 0.551$, $p = 0.008$), between N-MID and PYD ($r = 0.485$, $p = 0.001$) and lastly between P1NP and PYD ($r = 0.312$, $p = 0.047$) were found in the females only (Table 2). These markers are used to indicate the bone turnover. Some biomolecules are highly possible to have a positive correlation as they come from the similar molecules such as βCTx, PYD and DPD. Surprisingly, their correlations were found only in the female group [βCTx-PYD ($r = 0.365$, $p = 0.019$), DPD-PYD ($r = 0.567$, $p = 0.006$)]. These results could be caused by the wide ranges of the biomolecule levels in the males but not in the females. This research also found the correlation between UcOC and Vit D in the males ($r = 0.39$, $p = 0.04$) and the correlation between N-MID and Vit D in the females ($r = 0.347$, $p = 0.026$) (Table 5). These correlations can be explained that vitamin D affects the osteoblastic cells to produce osteocalcin[21]. Thus, there is the correlation between the osteocalcin and the vitamin D level. Finally, the correlations between UcOC and MGP ($r = 0.421$, $p = 0.006$) and between NMID osteocalcin and MGP ($r = 0.333$, $p = 0.033$) were found in the females only. These correlations are caused by both MGP and osteocalcin which are small protein synthesized by osteoblastic cells. MGP comprises of four types: 1) undercarboxylated MGP (ucMGP), 2) carboxylated MGP (cMGP), 3) dephospholylated MGP (dpMGP) and 4) phosphorylated MGP (pMGP)[14]. Although the kind of MGP in circulating blood could not be identified, the data showing the correlation between MGP and UcOC ($p = 0.006$) except the one of the correlation between MGP and Gla-OC ($p > 0.05$) were provided. Then, the researchers approximated that the found circulating MGP was ucMGP. This information is similar to the research by Cranenburg et al[21]. The levels of UcOC and ucMGP are vitamin K dependent. It is quite reasonable to assume that the correlations between MGP and UcOC and between MGP and N-MID are positive.

The benefits of the strong correlation will predict the status of the other if one item was checked that will alleviate the expense.

Potential conflicts of interest

None.

Table 3. Bone Marker Correlations in the Males

	N-MID		MGP		P1NP		βCTx		PYD		DPD		Vit D	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p
UcOC	0.282	0.146	0.193	0.326	0.322	0.094	0.471*	0.011*	0.256	0.189	0.287	0.264	0.390*	0.04*
N-MID	-	-	0.155	0.431	0.83*	0.00*	0.641*	0.00*	0.152	0.441	0.124	0.635	0.046	0.817
MGP	-	-	-	-	0.126	0.524	0.048	0.809	0.47*	0.012*	0.093	0.724	0.162	0.410
P1NP	-	-	-	-	-	-	0.657*	0.000*	0.123	0.532	0.044	0.866	0.107	0.589
βCTx	-	-	-	-	-	-	-	-	0.031	0.876	0.046	0.861	0.033	0.867
PYD	-	-	-	-	-	-	-	-	-	-	0.146	0.576	0.23	0.239
DPD	-	-	-	-	-	-	-	-	-	-	-	-	0.27	0.295

* r = correlation coefficient, p = p-value, The positive correlations between bone markers and vitamin D in the male group are shown by using '*'

Table 4. Bone Marker Correlations in the Females

	N-MID		MGP		P1NP		βCTx		PYD		DPD		Vit.D	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p
UcOC	0.110	0.495	0.421*	0.006*	0.039	0.809	0.105	0.515	0.318*	0.043*	0.551*	0.008*	0.160	0.319
N-MID	-	-	0.333*	0.033*	0.721*	0.000*	0.719*	0.000*	0.485*	0.001*	0.334	0.129	0.347*	0.026*
MGP	-	-	-	-	0.279	0.077	0.209	0.190	0.471*	0.002*	0.472*	0.026*	0.300	0.056
P1NP	-	-	-	-	-	-	0.632*	0.000*	0.312*	0.047*	0.11	0.626	0.239	0.132
βCTx	-	-	-	-	-	-	-	-	0.365*	0.019*	0.339	0.123	0.194	0.224
PYD	-	-	-	-	-	-	-	-	-	-	0.567*	0.006*	0.409*	0.008*
DPD	-	-	-	-	-	-	-	-	-	-	-	-	0.267	0.230

* r = correlation coefficient, p = p-value. The positive correlations between bone markers and vitamin D in the female group are shown by using '*'

Table 5. Summary of the moderate and strong correlation (r) between the type of bone markers in male and female

Correlation	Male	Female	p
PINP-NMID	0.83	0.721	0.001
CTx-NMID	0.641	0.719	0.001
CTx-PINP	0.657	0.632	0.001
MGP-PYD	0.47	*	0.012
UcOC-NMID	*	0.421	0.001
UcOC-DPD	*	0.551	0.008
UcOC-CTx	0.417	*	0.011

* = weak correlation

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รายงานเบื้องต้นความสัมพันธ์ระหว่างโบนมาร์เกอร์และวิตามินดี ในประชากรไทยปกติ

ชุติเพ็ญ บูรณะสินทรัพย์ , อรุณี แจ้งแสงทอง, ณรงค์ บุณยะรัตเวช

รายงานการศึกษา โบนมาร์เกอร์ชนิดต่างๆในกลุ่มประชากร 69 ราย เป็นชาย 28 ราย และหญิง 41 ราย ที่มีอายุ ตั้งแต่ 20-50 ปีที่สุขภาพแข็งแรง ผลการศึกษา พบว่าสหสัมพันธ์ระหว่างชนิดของโบนมาร์เกอร์ บางชนิดมีสหสัมพันธ์น้อยมากคือค่า อาร์ ต่ำกว่า 0.4 ส่วนที่มีสหสัมพันธ์กันตั้งแต่ปานกลางถึงมาก คือค่า 'อาร์' ตั้งแต่ 0.45 พบได้หลายคู่และมีความแตกต่างกันทางเพศด้วย ได้สรุปในตารางที่ 5 ประโยชน์ที่ได้รับทำให้ คาดคะเนระดับของโบนมาร์เกอร์อีกชนิดได้หากว่ามีความจำเป็นต้องตรวจชนิดเดียวอาจจะด้วยสาเหตุ การค่าใช้จ่ายหรือน้ำยาที่ใช้ตรวจไม่มีก็ได้