Special Article

Bone Markers 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*

Osteoporosis is a systemic skeletal disease characterized by low bone mineral density (BMD) and micro-architectural deterioration of the bone tissues resulting in bone fragility and susceptibility to fracture. It is caused by the decrease of bone formation and the increase of bone resorption. Both bone formation and resorption can be examined by the circulating biomolecules. The biomolecules using in determining the bone formation are composed of osteocalcin (OC) in form of N-terminal midmolecule fragment (N-MID) and undercarboxylated osteocalcin (UcOC). The other bone formation markers are matrix Gla protein (MGP) and N-terminal propeptide of Type I collagen (P1NP) whereas the biomolecules using in determining the bone resorption comprises of C-terminal cross-linking telopeptide of Type I collagen (betaCTx), collagen cross-links molecules which are pyridinoline (PYD) and deoxypyridinoline (DPD). Nevertheless, some vitamins such as vitamin D (Vit D) and some hormones e.g. parathyroid hormone (PTH) are also affected to the bone quality. To monitor and assess the bone mass, the normal values of bone markers as well as the relevant biomolecules are important and should be established. The researchers aimed to investigate the normal values of the interesting bone biomarkers and the relevant biomolecules in the adult volunteers.

Keywords: *Bone markers, Betacrosslap, Biomolecules, Normal values*

J Med Assoc Thai 2011; 94 (Suppl. 5): S96-S101
Full text. e-Journal: *http://www.mat.or.th/journal*

Bone is classified as active tissue. It possesses the coupling process between the formation and the resorption which is called bone turnover. In the healthy people, the rate of formation is equal to the resorption rate. However, in the osteoporosis patients, the rate of resorption is higher than the rate of formation resulting in the low bone mineral density (BMD). To predict the alteration of BMD, the biochemical markers of bone turnover can be used. In addition, the determination of vitamins and hormonal relevant with bone are helpful. Even though they provide the whole body rate of bone turnover, they can be useful for clinicians to monitor the bone mass of the osteoporosis patients.

The biochemical elements of bone markers can be classified into two major groups which are the biochemical markers of bone formation and of bone resorption.

Biochemical Markers of Bone Formation

There are a number of proteins which are produced and secreted to the circulation by osteoblasts. They are used as the markers of bone formation and can be detected in serum. They are composed of osteocalcin (OC), matrix Gla protein (MGP) and N-terminal propeptide of type I collagen (P1NP).

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

Osteocalcin (OC)

Osteocalcin or bone Gla protein is a small noncollagenous protein composed of 49 amino acid residues and 5.8 kDa in weight. It is produced by osteoblasts. Additionally, this biomolecule is highly specific for bone tissue. The newly synthesized OC is incorporated into the bone matrix. However, in the process of matrix synthesis, some OC is released into blood circulation. Although no intact OC is released during the bone resorption, fragments are released during the resorption and the formation. Among the fragments, N-terminal midmolecule fragment (N-MID) provides the best clinical information and it is easily detected by chemical reaction[1,2]. The structure of OC comprises of 3 glutamic acid residues which are post-translational carboxylated. After the carboxylation, it converts into carboxylated OC (Gla-OC)[3]. Nevertheless, OC in bone is not completely carboxylated and the serum level of the undercarboxy-

lated (UcOC) form increases due to age.

Matrix gla protein (MGP)

Matrix Gla protein is a small secretory protein which is firstly derived from bone. It is a vitamin K dependent protein composed of 84 amino acids containing 5γ-carboxyglutamic acid (Gla). The known function of Gla residues is to bind calcium both ions and crystal forms. In the restriction of vitamin K, undercarboxylated species of MGP are produced. However, the hypothesis is that the incomplete MGP carboxylation is common in the healthy people[4].

N-terminal propeptide of type I collagen (P1NP)

The collagen Type I is the most abundant protein synthesized by osteoblasts. After the secretion to the extracellular space, the C- and N-terminal propeptide (P1CP and P1NP) are cleaved off and released into the circulation. The presence of both procollagens in the circulation is directly related to the deposition of new Type I collagen. Although other tissues can also produce the Type I collagen that is not specific for bone, the amount of this production is quite small[1].

Biochemical Markers of the Bone Resorption

In the process of bone resorption, the osteoclasts destroy and break down many proteins from the bone tissues. These proteins are secreted to the circulation and used as the markers of bone resorption. They comprise of the resisted part of carboxy terminal of telopeptide called betacrosslap (betaCTx), pyridinoline (PYD) and deoxypyridinoline (DPD).

Telopeptide of type I collagen (β-CTx)

While the bone resorption is undergoing a change, the amino- and carboxy-terminal fragments of collagen are released into the circulation. They are called NTx and CTx respectively[1,5].

Pyridinolin (PYD) and deoxypyridinoline (DPD)

Pyridinoline (hydroxylysylpyridinoline) and Deoxypyridinoline (lysylpyridinoline) are collagen cross-links presented in the mature collagen. Their function is to stabilize the collagen's structure. PYD and DPD are found mainly in bone; however, PYD can also be found in other connective tissues. During the process of bone resorption, these cross-links are released into the serum. Definitely, they are useful for diagnosing and monitoring the osteoporosis and other metabolic bone diseases[6,7].

Vitamin and Hormone Relevant to Bone Mass

There are a vitamin and hormone that affect to the bone quality. They are vitamin D (Vit D) and parathyroid hormone (PTH).

Vitamin D (Vit D.)

Vitamin D plays an important role in the arrangement of mineral homeostasis. The active form of Vit D. stimulates calcium absorption from the intestine; regulates the bone resorption together with the formation. Vit D. deficiency leads to rickets in children and osteomalasia in adults. There are evidences[8,9] that supplements of Vit D. can reduce risks of bone fracture.

Parathyroid hormone (PTH)

Parathyroid hormone is the endocrine hormone produced by parathyroid glands. Its function is to regulate calcium homeostasis acting on bone, kidney and intestine. It is secreted in response to the reduction of calcium level. This action results in increasing the bone resorption. Additionally, in the case of Vit. D deficiency, secondary hyperparathyroidism causes high bone turnover leading to bone loss and subsequently increases fracture risks[10,11].

As the biochemical substances of bone markers and the relevant biomolecules can be used in monitoring the bone mass, the normal values of each parameter should be established. The aim of the present study is to publish the normal values of interesting bone biomarkers which are relevant with different genders.

Materials and Method

Subjects

The authors 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 CTx, P1NP, N-MID and relevant biomolecules which are Vit. D and PTH 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 manufacturer protocol.

For the UcOC analysis, 100 µl of serum and 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 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 96-well plate following with 200 µl of biotinylated synthetic MGP (tracer) and incubated at 4°C for over night. 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, approximately 200 ml of serum was filtrated through MWCO Spinfilter. Twenty-five microliters of filtrated serum, standard, low and high controls were added into the 96-well plate following with 75 µl of cold anti-PYD conjugated with 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 microliters of substrate was added into the well plate and incubated for 40 minutes. Finally, 100 µl of 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 ml 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 with 25 ml of total DPD assay buffer and 25 ml of total DPD base. The standard and control solutions were also neutralized with the total DPD buffer and the DPD base. The analysis procedure was started by adding 50 µl of 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 alkaline phosphatase was plated and incubated for 2 hours at 2-8°C. After removing the solution and doing the wash, 150 ml of substrate solution was added and incubated for 2 hours at RT. Finally, 100 µl of 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.

Results

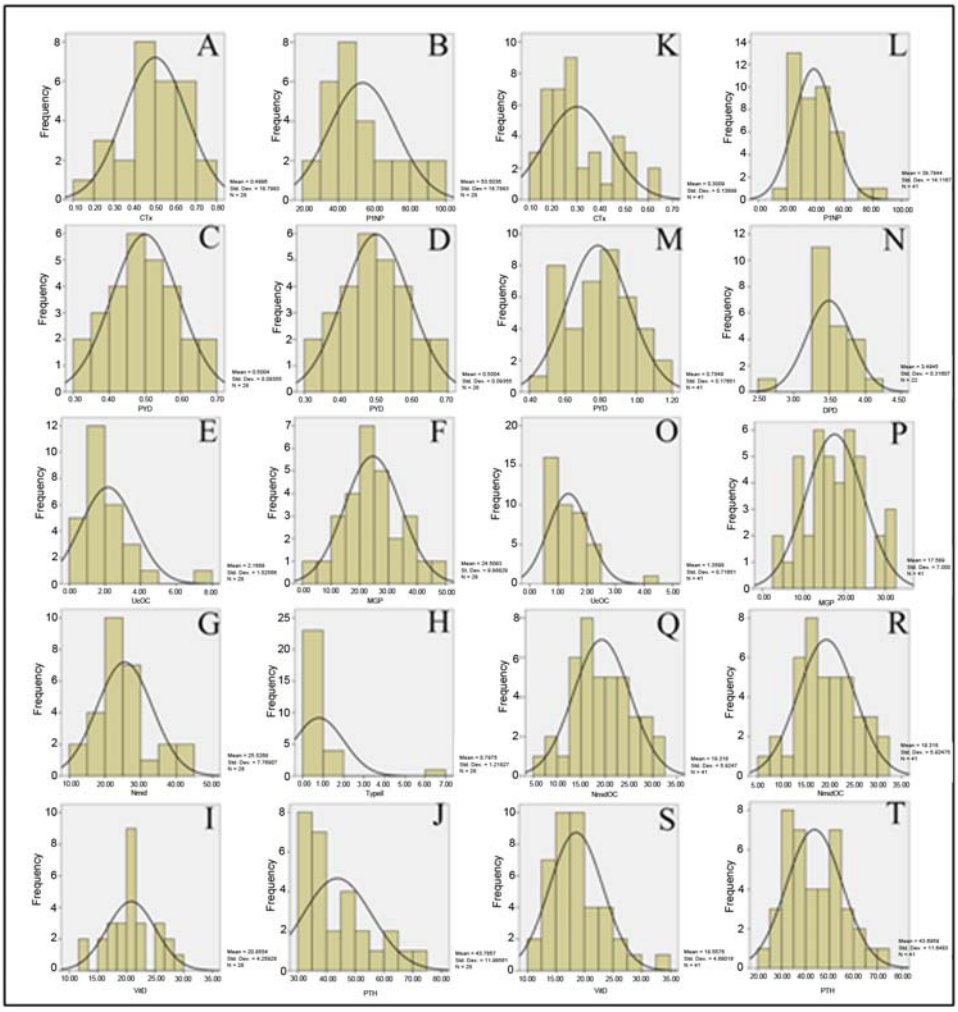
All analyzed biochemical bone markers are shown in the mean ± SD separated by male in Table 1 and female in Table 2.

Discussion

Osteoporosis is a silent disease characterized by the low level of the bone mineral density. It always occurs in the elderly especially in the menopause

Table 1. Biochemical Bone Markers in Male

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 (MGP), nMol/L	24.51 ± 9.89	28
N-terminal propeptide of type I collagen (P1NP), ng/ml	53.50 ± 18.8	28
Betacrosslap (betaCTx), ng/ml	0.50 ± 0.16	28
Pyridinolin (PYD), ng/ml	0.50 ± 0.09	28
Deoxypyridinoline (DPD), ng/ml	3.72 ± 0.42	17
Vitamin D (Vit D), ng/ml	20.86 ± 4.26	28
Parathyroid hormone (PTH), pg/ml	43.79 ± 11.99	28



A to J represent the histogram of biochemical bone markers in male whereas K to T represent the histogram of biochemical bone markers in female. A and K represent the histogram of CTx, B and L represent the histogram of P1NP, C and M represent the histogram of PYD, D and N represent the histogram of DPD, E and O represent the histogram of UcOC, F and P represent the histogram of MGP, G and Q represent the histogram of N-mid, H and R represent the histogram of Type II Col, I and S represent the histogram of Vit D, J and T represent the histogram of PTH.

Fig. 1 Histogram of Biochemical Bone Markers in Male and Female Volunteers

Table 2. Biochemical Bone Markers in Female

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
Betacrosslap (betaCTx), ng/ml	0.30 ± 0.14	41
Pyridinolin (PYD), ng/ml	0.78 ± 0.18	41
Deoxypyridinoline (DPD), ng/ml	3.49 ± 0.32	22
Vitamin D (Vit D), ng/ml	18.56 ± 4.68	41
Parathyroid hormone (PTH), pg/ml	43.70 ± 11.65	41

people. To diagnose this disease, the circulating biochemical bone markers and the relevant molecules are extremely useful. They are easy to analyze and can be used to monitor and predict the quality of bone mass. However, the normal values of these molecules in adults cannot indicate or predict the decreasing of bone quality. Besides, it is generally accepted that these markers are varied according to different nationals. The values of some markers in many countries such as Japan[12,13], China[14], Saudi Arabia[15] and USA[16] are investigated and used as the references, but there is not much information of these markers in Thailand. Narong[17,18] had reported the biochemical bone markers in Thai adults need to be more studied especially in female. Furthermore, the present study gave the information of male. Addition, some biomarkers, for example, serum PYD, DPD and MGP are quite the new data. They also are the helpful information for clinicians in determining, monitoring and predicting the bone mass of osteoporosis patients.

Potential conflicts of interest

None.

References

1. Swaminathan R. Biochemical markers of bone turnover. Clin Chim Acta 2001; 313: 95-105.
2. Ivaska KK, Hentunen TA, Vaaraniemi J, Ylipahkala H, Pettersson K, Vaananen HK. Release of intact and fragmented osteocalcin molecules from bone matrix during bone resorption in vitro. J Biol Chem 2004; 279: 18361-9.
3. Schurgers LJ, Cranenburg EC, Vermeer C. Matrix Gla-protein: the calcification inhibitor in need of vitamin K. Thromb Haemost 2008; 100: 593-603.
4. Bikle DD. Biochemical markers in the assessment of bone disease. Am J Med 1997; 103: 427-36.
5. Watts NB. Clinical utility of biochemical markers of bone remodeling. Clin Chem 1999; 45: 1359-68.
6. Hata K, Miura M, Fukumoto S, Matsumoto T. Assay of serum pyridinoline: a potential marker for bone resorption. Clin Chim Acta 1995; 235: 221-7.
7. Adamczyk M, Johnson DD, Reddy RE. Bone collagen cross-links: an efficient one-pot synthesis of (+)-pyridinoline and (+)-deoxypyridinoline. Tetrahedron: Asymmetry 2000; 11: 2289-98.
8. van Leeuwen JP, van den Bemd GJ, van Driel M, Buurman CJ, Pols HA. 24,25-Dihydroxyvitamin D (3) and bone metabolism. Steroids 2001; 66: 375-80.
9. Ikeda K, Ogata E. Modulation of bone remodeling by active vitamin D: its role in the treatment of osteoporosis. Mech Ageing Dev 2000; 116: 103-11.
10. de Paula FJ, Rosen CJ. Back to the future: revisiting parathyroid hormone and calcitonin control of bone remodeling. Horm Metab Res 2010; 42: 299-306.
11. Rosen CJ. Age-related osteoporosis and skeletal markers of bone turnover. In: Seibel MJ, Robins SP, Bilezikian JP, editors. Dynamics of bone and cartilage metabolism. 2nd ed. Burlington, MA: Academic Press; 2006: 671-87.
12. Iki M, Morita A, Ikeda Y, Sato Y, Akiba T, Matsumoto T, et al. Biochemical markers of bone turnover predict bone loss in perimenopausal women but not in postmenopausal women-the Japanese Population-based Osteoporosis (JPOS) Cohort Study. Osteoporos Int 2006; 17: 1086-95.
13. Iki M, Morita A, Ikeda Y, Sato Y, Akiba T, Matsumoto T, et al. Biochemical markers of bone turnover may predict progression to osteoporosis in osteopenic women: the JPOS Cohort Study. J Bone Miner Metab 2007; 25: 122-9.
14. Pi YZ, Wu XP, Liu SP, Luo XH, Cao XZ, Xie H, et al. Age-related changes in bone biochemical markers and their relationship with bone mineral density in normal Chinese women. J Bone Miner Metab 2006; 24: 380-5.
15. Ardawi MS, Maimani AA, Bahksh TA, Rouzi AA, Qari MH, Raddadi RM. Reference intervals of biochemical bone turnover markers for Saudi Arabian women: a cross-sectional study. Bone 2010; 47: 804-14.
16. de Papp AE, Bone HG, Caulfield MP, Kagan R, Buinewicz A, Chen E, et al. A cross-sectional study of bone turnover markers in healthy premenopausal women. Bone 2007; 40: 1222-30.
17. Bunyaratavej N, Kitimanon N, Boonthitikul S. Study of the level of biochemical bone markers: NMID osteocalcin and bone resorptive marker (beta CTx) in Thai women. J Med Assoc Thai 2001; 84 (Suppl 2): S560-5.
18. Bunyaratavej N, Kittimanon N. Study of procollagen type 1 nitrogenous propeptides(PINP) in reproductive female. J Med Assoc Thai 2005; 88(suppl 5):S27-S28.

รายงานการศึกษาโบนมาร์เกอร์ชนิดต่าง ๆ ในกลุ่มประชากรไทยที่ร่างกายปกติ

ชุติเพ็ญ ฐรณะสินทรัพย์, อรุณี แจ้งแสงทอง, ณรงค์ บุณยะรัตเวช

ศึกษาระดับ โบนมาร์เกอร์หลายชนิดได้แก่ UcOc, N-MID osteocalcin, MGP, PINP, betaCTx, PYD, DpD และวิตามินดี ในกลุ่มอาสาชายหญิงที่ร่างกายปกติจำนวน 69 ราย เพื่อเป็นค่าปกตินำไปเปรียบกับค่าประชากรในวัยอื่นซึ่งผลการศึกษาได้แสดงในตารางที่ 1 และ 2