Biochemical Markers of Bone Turnover in Angiographically-demonstrated Coronary Artery Disease Patients and Healthy Thais

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Abstract

The patients with coronary artery disease (CAD) were suffering from dyspnea. Physical activity of these patients was limited. Their lifestyle may be contributory factors for osteoporosis. Recent research has shown that biochemical markers may be used to predict future bone loss and identify individuals at risk for osteoporosis. Our objectives were to estimate reference ranges of bone markers in healthy Thais and to compare bone turnover between 105 healthy people and 118 CAD patients by using biochemical markers of bone formation and resorption. Mean values of bone markers in controls and patients were 22.9 ± 12.9, 21.6 ± 16.2 respectively for N-Mid osteocalcin and 0.45 ± 0.30, 0.47 ± 0.37 respectively for β-Crosslaps. There was no statistical difference of N-Mid osteocalcin (p=0.50) and β-Crosslaps (p=0.64) values between groups. Our data from this study suggested that that CAD patients have no higher risk for osteoporosis than healthy people.

Key word : Coronary Artery Disease, Bone Marker, Osteocalcin, Crosslaps

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Osteoporosis is common following cardiac transplantation and incidence of fracture is also high[1]. It is associated with low serum vitamin D metabolites, high level of bone resorption markers, exposure to glucocorticoids, and low serum testosterone in men[2-4]. However, mean bone mineral density may be low before the operation[5]. Low bone mass has been reported in patients with congestive heart failure and terminal heart disease [6-7]. Contributory factors in these patients include vitamin D deficiency, loop diuretics, dietary calcium deficiency, prerenal azotemia, hepatic congestion,

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and hypogonadism[6]. Lower pretransplant bone mineral density was associated with increasing fracture risk[5].

In case of coronary artery disease (CAD) patients, the physical activity is limited by dyspnea on exertion. This effect depends on the severity of disease. A severe CAD patient is confined to bed by dyspnea. Thus, CAD patients may be at risk for osteoporosis due to sedentary lifestyle and immobilization. Furthermore, recent studies have shown that noncollagenous bone matrix proteins are also found in atherosclerotic vessels and may regulate dystrophic calcification. Osteocalcin, the marker of bone formation, may contribute to vessel wall calcification after a thrombotic or hemorrhagic event[8]. In this study, we evaluated a group of patients with CAD to compare two biochemical bone markers of bone turnover, osteocalcin and β-Crosslaps, with healthy controls.

MATERIAL AND METHOD

Study populations

We studied 118 CAD patients who attended Her Majesty Cardiac Center at Siriraj Hospital between 1998 and 1999; 82 were men and 36 were women. Exclusion criteria included primary hyperparathyroidism, renal disease and long term steroid therapy. These patients consisted of 36 with unstable angina pectoris and 82 with stable angina pectoris. Coronary artery lesions were angiographically verified in all cases. One hundred and five blood donors were used as control subjects, 48 were men and 57 were women.

Biochemical Markers of Bone Turnover

Clotted blood was collected and centrifuged at 3000 g for 15 minutes. The serum was stored at −70°C until analysis.

Osteocalcin in serum was determined by Elecsys N-Mid osteocalcin immunoassay (Roche Diagnostics, Switzerland). This sandwich immunoassay uses two polyclonal antibodies specifically directed against epitopes on the N-Mid fragment and the N- terminal of osteocalcin molecule (biotinylated monoclonal N-Mid osteocalcin-specific antibody and monoclonal N-Mid osteocalcin-specific antibody labeled with ruthenium complex).

β-CTx in serum was determined by Elecsys β-Crosslaps serum immunoassay (Roche Diagnostics, Switzerland) which recognized linear β-8AA octapeptides (EKAHD-β-GGR). This immunoassay quantifies all type I collagen degradation fragments that contain the isomerized octapeptide β-8AA by using biotinylated monoclonal anti-β-Crosslaps antibody and monoclonal β-Crosslaps-specific antibody labeled with ruthenium complex.

Coronary Angiography

Coronary angiography was performed on 118 CAD patients. All of these had at least 1 diseased coronary artery, defined as a reduction of the vessel diameter of at least 50 per cent.

Statistical Analysis

The StatView (Abacus, USA) program was used for statistical analysis. Unpaired *t* test was employed for analysis. Values of $p<0.05$ were considered statistically significant.

RESULTS

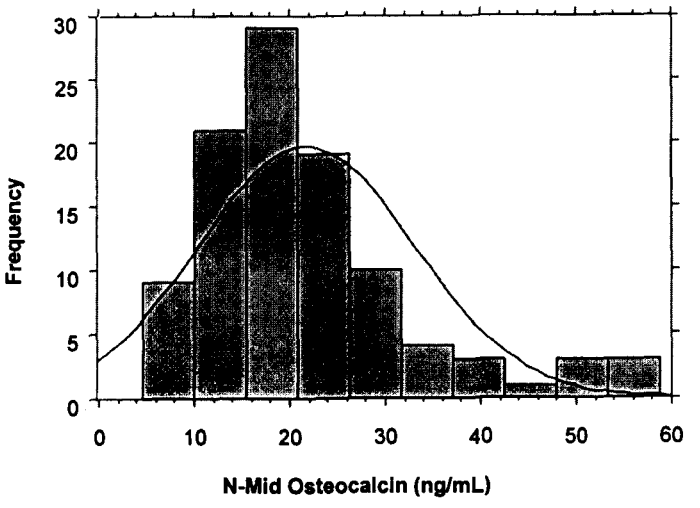
In the study of biochemical markers of bone turnover in 105 healthy adults, distributions of data were symmetrical as shown in Fig. 1. Values of N-Mid osteocalcin and β-Crosslaps were 22.9 ± 12.9 ng/mL and 0.45 ± 0.30 ng/mL respectively.

Differences of age, body mass index (BMI), osteocalcin, β-Crosslaps values in controls and CAD patients are shown in Table 1. Age and BMI of patients were slightly higher than healthy controls but not statistically significant ($p= 0.07$ and 0.16 respectively). Osteocalcin and β-Crosslaps were also not different ($p=0.50$ and 0.64 respectively). We compared these values between stable and unstable angina as shown in Table 2. There was no difference in any result.

DISCUSSION

Osteoporosis has numerous etiologies. The CAD patients may also have reduced bone mineral density due to activity limitation and exposure to diuretics in some cases. Inoue M, et al examined the biochemical markers of bone turnover in six healthy volunteers who participated in 120 days of bed rest. They found an increase in bone resorption markers and a decrease in bone formation markers during bed rest[9]. If osteoporosis is common in patients with CAD, all patients should be evaluated for osteoporosis.

Osteoporosis is characterized by abnormalities of bone remodeling that can be assessed by biochemical markers of formation and resorption. Recent research has shown that bone markers may



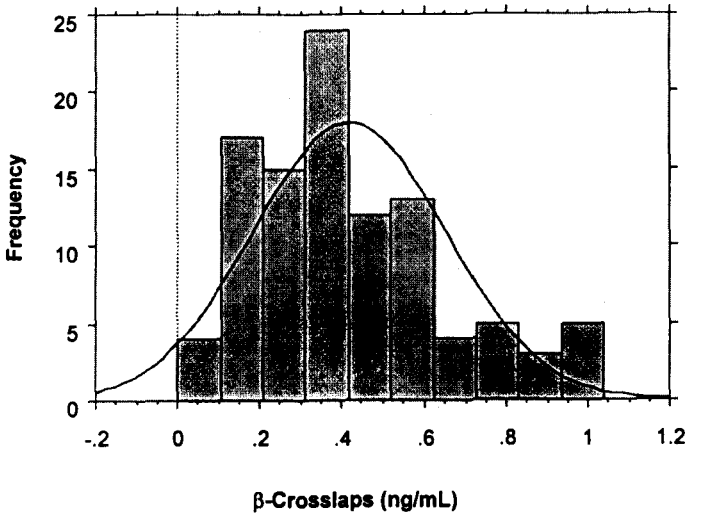


Fig. 1 Distributions of N-Mid osteocalcin, and β-Crosslaps values in healthy Thais.

Table 1. Age, body mass index, N-Mid osteocalcin, and β-Crosslaps values in controls and CAD patients.

	Controls (n= 105)	CAD Patients (n=118)	p Value
Age, year	59 ± 10	61 ± 9	NS
Body mass index	24.3 ± 4.4	25.5 ± 3.7	NS
N-Mid Osteocalcin, ng/mL	22.9 ± 12.9	21.6 ± 16.2	NS
β-Crosslaps,ng/mL	0.45 ± 0.30	0.47 ± 0.37	NS

NS = not significant

Table 2. Age, body mass index, N-Mid osteocalcin, and β-Crosslaps values in patients with stable and unstable angina.

	Stable Angina (n=82)	Unstable Angina (n=36)	p Value
Age,year	60 ± 10	62 ± 8	NS
Body mass index	25.8 ± 3.7	25.0 ± 3.7	NS
N-Mid Osteocalcin, ng/mL	21.2 ± 17.6	22.3 ± 12.4	NS
β-Crosslaps, ng/mL	0.46 ± 0.40	0.50 ± 0.29	NS

NS = not significant

also be used to identify individual at risk for osteoporosis, predict future bone loss and hip fracture, select therapy, and predict and monitor the therapeutic response. To date, the most valuable markers of bone formation and resorption are N-Mid osteocalcin and β-Crosslaps respectively.

The study of N-Mid osteocalcin, and β-Crosslaps in 105 healthy Thais has shown that age and sex seem to influence all values. These markers of bone formation and resorption are highest in elderly females due to lack of estrogen. Many studies have shown that other bone markers were also significantly changed in different menopausal status or serum estradiol (E2) levels such as urinary cross-linked N-telopeptide of type 1 collagen (NTx)[10], deoxypyridinoline (Dpd) and pyridinoline (Pyd)[11]. Pidetcha P, et al reported that urinary Dpd levels in postmenopausal Thai women was higher than premenopause but not statistically different[12]. This correlation can be clearly demonstrated in the study of association between sex hormones and biochemical markers of bone turnover by Peichl P, et al[13].

Elderly males have a higher value of bone markers than younger males but not statistically significant in our study. The mechanisms of bone loss in this group are still unknown. Some studies indicated that bone mineral density was not correlated to androgen levels in older men and must be associated with other factors[14,15]. Age-related bone loss may be the result of estrogen deficiency not just in postmenopausal women, but also in men[16].

The reference values of N-Mid osteocalcin in our study do not differ from values studied in a white population but β-Crosslaps values are higher. This finding may indicate interracial differences of β-Crosslaps. However, further study should be performed in large population to estimate reference ranges of bone markers in Thais.

Napal J, et al studied the serum level of osteocalcin in patients with acute myocardial infarction. The concentration of osteocalcin was significantly diminished. They were also studied in a group of patients with retinal detachment to exclude the immobilization as the cause of the decrease in serum osteocalcin and found that osteocalcin levels in this group were normal[17].

Our study, which compares the level of osteocalcin and β-Crosslaps between healthy controls and patients with angiographically-demonstrated CAD, shows no statistical difference in these markers of bone turnover. We assume that CAD patients have no higher risk for osteoporosis than normal people. However, some factors may disturb the results, including stability and diurnal variation of these bone markers[18]. We use N-Mid osteocalcin to avoid the stability problem of intact osteocalcin and use fasting blood specimen to reduce circardian rhythm of bone markers, especially β-Crosslaps[19]. Anyway, other bone markers should be studied to confirm the status of bone turnover in CAD patients because different markers reflect different events of sequence of bone remodeling.

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ดัชนีทางชีวเคมีของกระดูกในกลุ่มผู้ป่วยโรคหลอดเลือดหัวใจและกลุ่มคนไทยที่มีสุขภาพดี

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กิจกรรมต่างๆในชีวิตประจำวันของผู้ป่วยโรคหลอดเลือดหัวใจมักจะถูกจำกัดเนื่องจากอาการหอบเหนื่อยเมื่อออกแรง การดำรงชีวิตในลักษณะดังกล่าวเป็นระยะเวลานานอาจเป็นปัจจัยส่งเสริมให้เกิดโรคกระดูกพรุน ในปัจจุบันพบว่าดัชนีทางชีวเคมีของกระดูกมีประโยชน์ในการทำนายการสูญเสียเนื้อกระดูก และบ่งบอกความเสี่ยงในการเกิดโรคกระดูกพรุน การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อประเมินค่าอ้างอิงของดัชนีทางชีวเคมีของการสร้างและสลายเนื้อกระดูกโดยประมาณจากกลุ่มคนไทยที่มีสุขภาพดี 105 คน และนำมาเปรียบเทียบกับผู้ป่วยโรคหลอดเลือดหัวใจ 118 คน ค่าเฉลี่ยของดัชนีทางชีวเคมีของกระดูกของผู้ที่มีสุขภาพดีและผู้ป่วยโรคหลอดเลือดหัวใจเท่ากับ 22.9 ± 12.9, 21.6 ± 16.2 ตามลำดับสำหรับเอ็น-มิด ออสทีโอแคลซิน และ 0.45 ± 0.30, 0.47 ± 0.37 ตามลำดับสำหรับเบต้า-ครอสแล็พซ์ ซึ่งไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ จากการศึกษาครั้งนี้พอจะสรุปได้ว่าผู้ป่วยโรคหลอดเลือดหัวใจไม่ได้มีความเสี่ยงต่อการเกิดโรคกระดูกพรุนมากกว่าคนทั่วไป

คำสำคัญ : โรคหลอดเลือดหัวใจ, ดัชนีทางชีวเคมีของกระดูก, ออสทีโอแคลซิน, ครอสแล็พซ์

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