Association Between Estrogen Receptor Concentration in Breast Cancer Tissue and Bone Mineral Density

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Abstract

Both bone and the breast are major target tissues of estrogen actions. The biological actions of estrogen depend on the interaction between estrogen and estrogen receptors (ER) in the target tissues. Therefore, ER concentration in tissues such as breast cancer might be associated with the amount of bone mass. The present study was aimed to examine whether there is a relationship between ER concentration in breast cancer tissue (ER-BCA) and bone mineral density (BMD). Forty-seven pre-menopausal and 34 post-menopausal women with newly diagnosed breast cancer were studied. The ER-BCA ranged from 0 to 339 fmol/mg cytosol protein (mean $\pm$ SD = 68.6 $\pm$ 97.0). Pearson's correlation analyses showed that ER-BCA negatively correlated to BMD of the spine ($r = -0.251$, $p = 0.024$), forearm ($r = -0.341$, $p = 0.002$), hip ($r = -0.373$, $p = 0.001$) and total body ($r = -0.317$, $p = 0.004$) in all 81 women. In 47 pre-menopausal women, the ER-BCA negatively correlated to the hip ($r = -0.455$, $p = 0.001$) and total body ($r = -0.395$, $p = 0.006$) but not to the spine and forearm BMD. Whereas, in 34 post-menopausal women, the ER-BCA negatively correlated to forearm BMD ($r = -0.399$, $p = 0.019$). Stepwise multiple regression analyses showed that the ER-BCA independently correlated to hip BMD in all 81 women ($r = -0.373$, $p < 0.01$) and in pre-menopausal women ($r = -0.486$, $p < 0.001$) and independently correlated to forearm BMD in post-menopausal women ($r = -0.399$, $p < 0.05$). The results of this study suggest that the presence of high estrogen receptor concentration in breast cancer tissue might induce a deleterious effect on bone mass particularly in pre-menopausal women.

Key word : Breast Cancer, Bone Mineral Density, Estrogen Receptor Concentration, Pre-Menopause, Post-Menopause

SRIUSSADAPORN S, SRIMUNINNIMIT V, PLOYBUTR S, et al
J Med Assoc Thai 2002; 85: 327-333

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Cumulative exposure to endogenous and exogenous estrogens in women are closely related to a higher bone mass[1-3], a lower risk of fractures[4] and a higher risk of primary breast cancer[5,6]. In addition, recent studies have shown that post-menopausal women with higher bone mass possess a higher risk of breast cancer[7,8]. As the biological actions of estrogen depend on circulating estrogen level, the concentration of estrogen receptor (ER) and the interaction between estrogen and ER in the tissues[9], the concentration of ER in tissues such as breast cancer might, therefore, be related to the amount of bone mass. Nielsen et al[10] first showed that women with ER-positive breast cancer had a higher forearm bone mineral content than did age-matched women with ER-negative breast cancer. However, the association between ER concentration in breast cancer tissue and the amount of bone mass could not be confirmed by their subsequent study [11]. As far as is known, there is no other published study on this issue. The present cross-sectional study was aimed to examine whether there is an association between amount of bone mass and ER concentration in breast cancer tissue (ER-BCA).

SUBJECTS AND METHOD

Subjects

Forty-seven pre-menopausal and 34 post-menopausal women with newly diagnosed stage I and stage II primary breast cancer were studied. None of them had bone metastasis, hypercalcemia, current and past history of intensive calcium and vitamin D supplements, oral contraceptive pill administration, estrogen replacement therapy, cigarette smoking, alcohol consumption or other conditions known to affect tissue ER regulation or calcium and bone metabolism. The diagnosis of breast cancer was established by histopathological study.

Estrogen receptor assay

After resection of breast cancer tissues, ER-BCA were measured by enzyme-immunoassay method using a commercial kit available from Abbot Laboratories, USA.

Bone mass measurement

The amount of bone mass was expressed as bone mineral density (BMD). BMD of the forearm, hip, lumbar spine 2-4 and total body were measured by dual energy X-ray absorptiometry using an EXPERT-XL (Lunar Corporation, USA). A coefficient of variation in BMD measurement of each skeletal site was 0.4 per cent. A difference in BMD value of greater than 0.4 per cent was considered clinically significant.

Statistical analyses

Data were expressed as mean $\pm$ standard deviation. Statistical analyses were performed using Statistical Packages for the Social Sciences version 9.0. Student's *t*-test was used to compare the continuous data between two groups. Pearson' s correlation analyses were used to examine the correlation between BMD and ER-BCA. Stepwise multiple regression analyses were performed to examine whether the ER-BCA independently related to BMD. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

The patients' characteristics are shown in Table 1. Of the total 81 cases, the ER-BCA ranged from 0 to 339 fmol/mg cytosol protein (68.6 $\pm$ 97.0). There was no significant difference in the concentrations of ER-BCA between pre-menopausal and post-menopausal women. The pre-menopausal group had higher BMD of all skeletal sites than did the post-menopausal group as predicted.

Pearson's correlation analyses showed that ER-BCA negatively correlated to BMD of the spine ($r = -0.251$, $p = 0.024$), forearm ($r = -0.341$, $p = 0.002$), hip ($r = -0.373$, $p = 0.001$) and total body ($r = -0.317$, $p = 0.004$) in all 81 women (Table 2). When post-menopausal and pre-menopausal women were analyzed separately, negative correlation between ER-BCA and BMD of different skeletal sites was still present. In 47 pre-menopausal women, the ER-BCA negatively correlated to the hip ($r = -0.455$, $p = 0.001$) and total body ($r = -0.395$, $p = 0.006$) but not to the spine and forearm BMD. Whereas, in 34 post-menopausal women, the ER-BCA negatively correlated to forearm BMD ($r = -0.399$, $p = 0.019$).

Stepwise multiple regression analyses showed that the ER-BCA independently correlated to hip BMD in all 81 women ($r = -0.373$, $p < 0.01$) and in pre-menopausal women ($r = -0.486$, $p < 0.001$) and independently correlated to forearm BMD in post-menopausal women ($r = -0.399$, $p < 0.05$). The degree of correlation was higher in pre-menopausal women. Fig. 1 shows scatter plots of the con-

Table 1. Characteristics of women with primary breast cancer.

	All cases		Pre-menopause		Post-menopause	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Cases	81		47		34	
Age (years)	29.0 - 67.0	49.0 ± 8.8	29.0 - 50.0	42.9 ± 5.5	51.0 - 67.0	57.4 ± 4.3
ER concentration in breast cancer tissue (fmol/mg cytosol protein)	0.0 - 339.0	68.6 ± 97.0	0.0 - 339.0	56.4 ± 83.5	0.0 - 339.0	85.4 ± 112.2
BMD (g/cm^2)						
Forearm	0.441 - 1.032	0.827 ± 0.098	0.698 - 1.003	0.860 ± 0.062	0.441 - 1.032	0.782 ± 0.121*
Hip	0.404 - 1.309	0.756 ± 0.160	0.549 - 1.309	0.819 ± 0.145	0.404 - 0.992	0.668 ± 0.138*
Spine	0.748 - 1.553	1.117 ± 0.156	0.905 - 1.553	1.180 ± 0.129	0.748 - 1.308	1.031 ± 0.150*
Total body	0.957 - 1.310	1.137 ± 0.08	0.987 - 1.310	1.160 ± 0.071	0.957 - 1.253	1.107 ± 0.082*

* $p < 0.01$ *vs* pre-menopause according to the Student's *t*-test
ER = estrogen receptor, BMD = bone mineral density

Table 2. Correlation between estrogen receptor concentration in breast cancer tissue and bone mineral density.

	Forearm BMD	Hip BMD	Spine BMD	Total body BMD
All 81 cases	-0.341**	-0.373**, ++	-0.251*	-0.317**
Pre-menopause 47 cases	-0.159	-0.486**, +++	-0.249	-0.395*
Post-menopause 34 cases	-0.399*, +	-0.240	-0.172	-0.192

Pearson' s correlation analyses (2-tailed): * $p < 0.05$, ** $p < 0.01$
Stepwise multiple regression analyses: + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$
BMD = bone mineral density

centration of ER-BCA *versus* BMD of skeletal sites that had significant correlation to the ER-BCA as determined by stepwise multiple regression analyses.

DISCUSSION

Recent studies in post-menopausal women have shown a direct association between bone mass and risk of breast cancer[7,8]. As bone and breast are the major target tissues of estrogen, it has been proposed that cumulative exposure to estrogen may play a role in the mechanism underlying this association[7,8]. Since the biological actions of estrogen depend on the interaction between estrogen and ER in target tissues[9], it is likely that ER-BCA might positively relate to bone mass. Nielsen et al[10] first demonstrated that women with ER-positive breast cancer had a higher forearm bone mineral content than age-matched women with ER-negative breast cancer. However, the association between ER-BCA and amount of bone mass could not be confirmed by their subsequent study[11].

Compared to the previous studies of Nielsen et al[10,11], the present study showed a different result that ER-BCA had significantly negative correlation to BMD. Women with a higher ER-BCA had a lower BMD than women with a lower ER-BCA. The negative correlation between ER-BCA and BMD as observed in the present study does truly exist because of the following reasons. Firstly, the ER-BCA had consistently negative correlation to BMD of all skeletal sites either when studied in all women or studied separately in post-menopausal and pre-menopausal women, though significant correlation was observed at some but not all skeletal sites. Secondly, stepwise multiple regression analyses were performed to correct all confounding factors possibly affecting the correlation between ER-BCA and BMD and the result showed that ER-BCA was independently associated with BMD of the forearm in post-menopausal women and BMD of the hip in pre-menopausal women.

The mechanism underlying the negative correlation between ER-BCA and BMD observed in this study is not clearly known. The roles of tissue ER function and concentration on bone metabolism and bone mass have been investigated. Smith et al

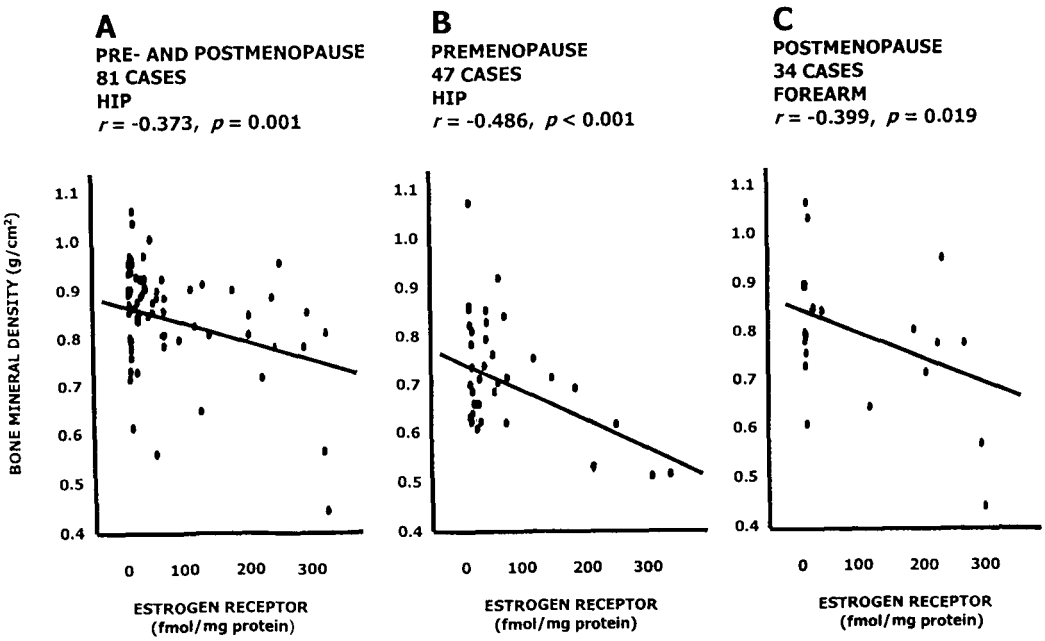

Fig. 1. Scatter plots of estrogen receptor (ER) concentration in breast cancer tissue *versus* bone mineral density of skeletal sites that had significant correlation to ER concentration determined by stepwise multiple regression analyses.

(12) have reported that a man who was homozygous for an inactivating point mutation in the estrogen receptor-α gene demonstrated estrogen resistant, increased bone turnover and severe osteoporosis. Their findings suggest that tissue estrogen receptor is essential in the regulation of bone metabolism and bone mass. Due to the difficulty in the measurement of ER in bone, previous studies on the association between tissue ER concentration and bone mass have used tissues other than bone of which the ER concentration can be easily and accurately measured. However, these studies revealed an inconsistent relationship between bone mass and ER concentration in extra-skeletal tissues such as vaginal epithelial cells(13) and uterine cervical tissue(14). Bartizal et al(13) found a decreased uptake of (^{3}H) estradiol by vaginal epithelial cells in osteoporotic compared with nonosteoporotic subjects, suggesting that a decreased ER number in extra-skeletal tissue such as vaginal epithelial cells might reflect a decreased ER number and decreased estrogen actions in bone. In contrast, Davidson et al(14) found that there was no significant difference in the concentration of ER in uterine cervical tissue between women with and without post-menopausal osteoporosis. They concluded that abnormal binding of sex steroid to ER in uterine cervical tissue is not the major factor accounting for decreased bone mass in women with post-menopausal osteoporosis. The inconsistent results of the studies on the relationship between ER concentration in various extra-skeletal tissues and bone mass could partly be explained by the differences in study designs, number and characteristics of subjects, methods used for measurement of tissue ER concentration and methods of statistical analyses among the studies. In addition, as the ER protein concentration and gene expression are different among various tissues(15), the ER concentration in extra-skeletal tissue might or might not represent the ER concentration in tissues throughout the whole body including bone. Therefore, the ER-BCA might not reflect the ER concentration in bone of women with breast cancer. A study by Nguyen-Pamart M et al(16) showed that women with breast cancer have increased bone resorption, irrespective of whether or not they have bone metastasis. Their study indicates that there might be an interaction between breast cancer tissue and bone cell functions. These lines of evidence suggest that the negative correlation between ER-BCA and BMD observed in this study might be due to an interaction between bone cell function and the ER in breast cancer tissue rather

than the ER in bone tissue of women with breast cancer. An *in vitro* study by Valentin-Opran et al[17] showed that ER-positive human breast cancer cell line (MCF-7) releases more bone resorbing activity such as prostaglandin E_2 and induces more bone resorption in response to estrogens than the ER-negative human breast cancer cell line (MDA-231). Regarding Valentin-Opran's study, negative correlation between ER-BCA and BMD observed in this study could be explained by the following proposed mechanism. Breast cancer tissue with high ER concentration might release more bone resorbing activity that might subsequently induce more bone loss than the breast cancer tissue with negative or low ER concentration. Therefore, women with higher ER-BCA would have lower bone mass as compared to the women with lower ER-BCA. The stronger correlation observed in pre-menopausal women might be due to a higher response of breast cancer tissue with high ER number to a higher circulating estrogen level during pre-menopausal compared to the post-menopausal period. In addition, there are other factors such as post-menopausal estrogen deficiency that might induce bone loss more strongly than breast cancer tissue. The present finding supports the clinical observations that women with ER positive breast cancer have a higher risk of bone metastasis [18] and bone resorption[19]. Further studies on bone resorbing activity such as prostaglandins and bone turnover status either by measuring biochemical markers of bone turnover or bone histomorphometry at first diagnosis of primary breast cancer are required to verify the mechanism underlying the negative correlation between ER-BCA and bone mass.

In conclusion, the present study has demonstrated that ER-BCA negatively correlates to bone mass. The negative correlation is stronger during the pre-menopausal period. The results of this study suggest that the presence of high estrogen receptor concentration in breast cancer tissue might possess a deleterious effect on bone mass particularly in pre-menopausal women. The present observation has opened an issue of interest that the interactions between estrogen receptor concentration in breast cancer tissue and bone cell functions should be further studied.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Meta Poojaroenchanachai for his great help in preparing this manuscript.

(Received for publication on August 21, 2001)

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ความสัมพันธ์ระหว่างจำนวนจำนวนเอ็สโตรเจนรีเซ็พเตอร์ที่เนื้อเยื่อมะเร็งเต้านมกับปริมาณมวลกระดูก

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เอ็สโตรเจนเป็นฮอร์โมนที่มีบทบาทสำคัญในการควบคุมปริมาณมวลกระดูกและการเกิดมะเร็งเต้านม เนื่องจากเอ็สโตรเจนออกฤทธิ์ทางชีวภาพโดยการจับกับเอ็สโตรเจนรีเซ็พเตอร์ที่เนื้อเยื่อเป้าหมาย จำนวนเอ็สโตรเจนรีเซ็พเตอร์ที่เนื้อเยื่อเป้าหมายจึงมีผลต่อการออกฤทธิ์ของเอ็สโตรเจน การศึกษานี้มีจุดประสงค์เพื่อศึกษาความสัมพันธ์ระหว่างจำนวนเอ็สโตรเจนรีเซ็พเตอร์ที่เนื้อเยื่อมะเร็งเต้านมกับปริมาณมวลกระดูก ผู้ป่วยสตรีซึ่งมีมะเร็งเต้านมที่เพิ่งได้รับการวินิจฉัยใหม่จำนวน 81 ราย (47 ราย อยู่ในวัยก่อนหมดระดู และ 34 ราย อยู่ในวัยหลังหมดระดู) ได้รับการตรวจวัดจำนวนเอ็สโตรเจนรีเซ็พเตอร์ที่เนื้อเยื่อมะเร็งเต้านมโดยวิธีเอ็นซัยม์อิมมูโนแอสเสย์ และปริมาณมวลกระดูกโดยการวัดค่าความหนาแน่นเกลือแร่ที่กระดูกสันหลัง, กระดูกสะโพก, กระดูกเรเดียสส่วนปลายล่าง และกระดูกรวมทั้งตัว ผลการศึกษาพบว่า ผู้ป่วยทั้งหมด 81 รายมีจำนวนเอ็สโตรเจนรีเซ็พเตอร์ที่เนื้อเยื่อมะเร็งเต้านมระหว่าง 0–339 เฟ็มโตโมล/1 มก. ซัยโตซอลโปรทีน (เฉลี่ย 68.6 ± 97.0) ซึ่งมีความสัมพันธ์ทางลบกับค่าความหนาแน่นเกลือแร่กระดูกสันหลัง ($r = -0.251$, $p = 0.024$), กระดูกเรเดียสส่วนปลายล่าง ($r = -0.341$, $p = 0.002$), กระดูกสะโพก ($r = -0.373$, $p = 0.001$) และกระดูกรวมทั้งตัว ($r = -0.317$, $p = 0.004$) ในผู้ป่วยวัยก่อนหมดระดู จำนวนเอ็สโตรเจนรีเซ็พเตอร์ที่เนื้อเยื่อมะเร็งเต้านมมีความสัมพันธ์ทางลบกับค่าความหนาแน่นเกลือแร่กระดูกสะโพก ($r = -0.455$, $p = 0.001$) และกระดูกรวมทั้งร่างกาย ($r = -0.395$, $p = 0.006$) ส่วนในผู้ป่วยวัยหลังหมดระดู จำนวนเอ็สโตรเจนรีเซ็พเตอร์ที่เนื้อเยื่อมะเร็งเต้านมมีความสัมพันธ์ทางลบกับค่าความหนาแน่นเกลือแร่กระดูกเรเดียสส่วนปลายล่าง ($r = -0.399$, $p = 0.019$) ผลการศึกษาดังกล่าวแสดงว่าจำนวนเอ็สโตรเจนรีเซ็พเตอร์ที่เนื้อเยื่อมะเร็งเต้านมมีความสัมพันธ์ทางลบกับปริมาณมวลกระดูก ความสัมพันธ์ดังกล่าวมีความชัดเจนในวัยก่อนหมดระดูมากกว่าในวัยหลังหมดระดู เนื้อเยื่อมะเร็งเต้านมที่มีจำนวนเอ็สโตรเจนรีเซ็พเตอร์มากอาจมีผลทำให้ปริมาณมวลกระดูกลดลงได้โดยเฉพาะอย่างยิ่งในสตรีวัยก่อนหมดระดู

คำสำคัญ : Breast Cancer, Bone Mineral Density, Estrogen Receptor Concentration, Pre–Menopause, Post–Menopause

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จดหมายเหตุทางแพทย์ ฯ 2545; 85: 327–333

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