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New evidence that tamoxifen does not induce osteoporosis: a nuclear activation analysis and absorptiometry study

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Abstract. The possibility of increased risk for osteoporosis in breast cancer patients treated with tamoxifen was investigated. 26 patients aged 41–65 years without skeletal metastases were studied. All patients were treated with 20 mg/d tamoxifen for a mean time of 22 months. The data obtained by *in vivo* neutron activation analysis of the phosphorus content in hands, were supplemented with data obtained by single photon absorptiometry in the forearm and radiographic morphometry. Comparison of the data with that of age and sex matched normal controls showed that breast cancer patients treated with tamoxifen are not prone to osteoporosis.

Breast cancer is one of the most common malignant tumours in women. A significant proportion of these carcinomas are oestrogen dependent tumours, therapeutically managed by several hormonal manipulations (Wittliff, 1984). Tamoxifen, an anti-oestrogen compound, is commonly used either as an adjuvant therapy, or in the management of metastatic disease (Ingle, 1984). Recently, the role of tamoxifen as a potential promoter for osteoporosis became a subject of experimental and clinical investigations (Gotfredsen et al, 1984; Love et al, 1988; Fentiman et al, 1989; Turken et al, 1989). Primary osteoporosis is characterized by decreased bone mass and increased susceptibility to fractures in the absence of other recognizable causes of bone loss. Early menopause and the administration of certain drugs are accepted as pre-disposing risk factors (Riggs & Melton, 1986).

The goal of the present study was to determine whether the administration of tamoxifen in breast cancer patients was associated with significant alterations of the bone minerals. *In vivo* phosphorus measurements were applied in breast cancer patients by neutron activation analysis (NAA) for the first time in the literature. The data from this technique were supplemented with data obtained by two conventional techniques, single photon absorptiometry (SPA) and radiographic morphometry. NAA provided data on phosphorus, and SPA data indirectly on both calcium and phosphorus, with a preponderance of calcium.

Materials and methods

Patients and normal controls

A total of 26 female breast cancer patients with a mean age of 53 years (range 41–65) were studied. All patients underwent modified radical mastectomy and were ambulatory with good performance status. None of these patients had bone metastases. Three patients were pre-menopausal, 21 post-menopausal and two peri-menopausal (menopause duration less than 2 years). Mean duration of menopause was 80 months (range 15–215 months). Natural cessation of menses occurred in 17 patients, while early menopause had been induced either surgically or chemically in another six patients. All patients were receiving tamoxifen 20 mg daily for a mean time of 22 months (range 12–45 months). Tamoxifen was administered as an adjuvant therapy in 22 patients and as treatment for advanced disease in the remaining four women. Among the latter, one had both supraclavicular lymph node and lung metastases, one had mediastinal involvement, one had only parenchymal lung lesions, while the last patient had ipsilateral axillary metastases. Nine patients had previously received chemotherapy, mainly as an adjuvant treatment.

Blood samples were obtained from all patients for the determination of serum concentrations of calcium, phosphorus, proteins, alkaline and acid phosphatase by standard procedures.

A group of 50 healthy female volunteers matched to patients for age, bodily build and full-term pregnancies was used as a control (Table I). None of them had a history of clinical conditions related to bone disease or evidence of osteopenia and they were also not users of oral contraceptives or medications related to bone mineral alterations. Lateral radiographs of the spine were required for normal subjects older than 55 years in order to investigate radiological signs of osteopenia.

Techniques

Partial body neutron activation analysis (PBNAA). PBNAA was applied for measuring hand bone phosphorus (HBP). About 90% of the body's phosphorus is

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Table I. Characteristics of patients and normal controls

Group	Patients	Controls	p
Number of subjects	26	50	
Age (years)	53.7±7.2	53.0±7.8	0.69
Mass (kg)	70.9±10.3	69.4±10.4	0.52
Height (cm)	153.6±5.6	155.2±6.8	0.31
Full-term pregnancies	1.8±0.9	1.7±0.9	0.60

Mean values±standard deviation.

found in the skeleton. Body sections, such as the hands, can be selected to measure phosphorus, where the amount of soft tissue present is small compared with that of bone. The technique is based on the detection of the delayed γ-rays emitted from ^{28}Al, which is produced via the $^{31}P(n,\alpha)^{28}Al$ fast neutron reaction. The 4 min irradiation of the hands with two ^{241}Am-Be neutron sources, is followed by a 4 min counting of the induced activity (Glaros et al, 1987). The 1.78 MeV photons emitted by ^{28}Al are counted with the hands sandwiched in contact with two cylindrical 20.3 cm × 10.2 cm NaI(Tl) detectors. The phosphorus content was expressed as the number of detected 1.78 MeV photons and no attempt was made to correlate quantitatively the ^{28}Al counts with the phosphorus mass because of the potential presence of large systematic errors. The short term *in vivo* precision (coefficient of variation) was found to be 2.9% for 50-year-old healthy women. This was found by measuring twice 30 normal volunteers within a period of a few days. The absorbed dose at any location of the skin palm ranges between 0.5 and 2.0 mGy. 80% of this dose is due to fast neutrons and the rest is due to photons. The dose equivalent does not exceed 0.2 mSv at any location in the trunk (Kalef-Ezra et al, 1990). The effective dose equivalent of the examination is about 100 μSv and the radiological risk factor of the examination is of lower magnitude than that of a typical lateral radiological examination of the lumbar spine.

Single photon absorptiometry (SPA). A modified version of the Cameron–Sorensen technique (Cameron & Sorensen, 1963) was applied for the measurement of bone mineral content (BMC) over the predominantly cortical distal third of the radius and the ulna with a prototype SPA system. The technique is based on the attenuation of a collimated γ-ray beam (59.5 keV from ^{241}Am) with the forearm submerged in water. The entrance skin dose was 40 μGy in a 6 cm^2 area.

Radiographic morphometry. Mammographic films were used for the postero-anterior radiographs of the hands. The Barnett-Nordin index (BNI) was determined at the midshaft of the right second metacarpal using a magnifier with a scale division of 0.1 mm. BNI values below 43 were assumed to be abnormal (Aloia et al, 1976). The entrance skin dose was 350 μGy in an area of about 120 cm^2.

Lateral radiographs of the patients' spines were obtained and read blindly. Vertebral compression, wedge-shape deformity, biconcavity and vertical trabeculation were investigated (Reinbold et al, 1986).

Data analysis

The normal control subjects were divided into five subgroups (41–45, 46–50, 51–55, 56–60 and 61–65 years) of 10 women each. The patients' data were expressed as the percentage of the mean normal values for the corresponding 5 year interval. Provided that the data were normally distributed, statistical analysis was performed using a t-test.

Results

No subject was classified as osteopenic in the spine according to conventional radiological criteria. Pathological BNI was found in the right second metacarpal in a 52-year-old patient who exhibited normal BNI in the left hand and early natural menopause at the age of 40 years. No case was found with a HBP value in the low 5% percentile of age matched controls (Table II). The age corrected HBP in the patient's group was 99.5%±9.6% (mean value±1 SD) and the t-test between the patients and the controls provided no evidence of difference in the mean values between the two populations ($p = 0.84$). The age corrected forearm BMC in the patient's group was 98.8%±12.5%, however, the t-test failed to provide evidence of the difference in the mean values between the two populations ($p = 0.65$).

The two groups reported similar dairy consumption, physical activity, full-term pregnancies, alcohol consumption and smoking habits, but differed in menopausal status. Measurements of serum, concentrations of calcium, phosphorus, proteins, alkaline and acid phosphatase were within normal limits in all patients.

We further investigated the influence of the following clinical parameters: duration of tamoxifen administration; prior chemotherapy; cause of menopause and presence of metastatic disease (Table III). Analysis of the data demonstrated that:

(a) the linear correlation of HBP and BMC values with the duration of tamoxifen administration ($r = 0.04$, $p > 0.1$ and $r = 0.24$, $p > 0.1$, respectively) indicated that the data failed to provide evidence of bone mineral loss.
(b) Comparison of the HBP and BMC values of those patients who had received chemotherapy with the

Table II. Age-adjusted data on the bone mineral status in the upper extremities by *in vivo* neutron activation analysis of phosphorus and single photon absorptiometry

Group	Controls	Patients	p
Hand bone phosphorus	100.0±9.9	99.5±9.6	0.84
Bone mineral content	100.0±9.3	98.8±12.5	0.65

Mean values±standard deviation expressed as percent of matched controls.

Table III. Age-adjusted data on the bone mineral status in the upper extremities in subgroups of patients

Duration of treatment (months)	12–20	21–45	*p*
Number of patients	13	13	
Hand bone phosphorus	99.4 ± 8.5	99.8 ± 10.6	0.91
Bone mineral content	95.2 ± 14.0	102.4 ± 10.1	0.15
CMF chemotherapy	no	yes	*p*
Number of patients	17	9	
Hand bone phosphorus	99.0 ± 7.8	100.5 ± 12.9	0.73
Bone mineral content	100.3 ± 14.4	96.1 ± 7.8	0.44
Induced menopause	no	yes	*p*
Number of patients	20	6	
Hand bone phosphorus	100.4 ± 9.9	96.5 ± 8.5	0.40
Bone mineral content	100.5 ± 13.6	93.1 ± 9.6	0.21
Metastatic disease	no	yes	*p*
Number of patients	22	4	
Hand bone phosphorus	98.3 ± 8.7	106.8 ± 12.8	0.14
Bone mineral content	98.9 ± 13.1	98.4 ± 10.4	0.94

Mean values ± standard deviation expressed as percent of matched controls.

rest of the patients showed no statistically significant difference ($p = 0.7$ and $p = 0.4$, respectively).

(c) The patients with regular menses or natural menopause (age at menopause 49.3 ± 4.3 years) had mean HBP values similar ($p = 0.9$) to those of matched controls and to those six patients with iatrogenically induced menopause ($p = 0.4$). The BMC data on this group did not provide any evidence of difference from either the controls ($p = 0.8$) or the patients with induced menopause ($p = 0.2$). The HBP and BMC data in the small group of patients with iatrogenically induced menopause (age at menopause 44 ± 5 years) were in the normal limits.

(d) The HBP and BMC values of the patients free of metastatic disease showed no significant difference from the matched controls ($p = 0.5$ and $p = 0.7$, respectively). In addition, the HBP and BMC data in four patients with advanced disease were in the normal limits.

Discussion

The amount of calcium and phosporus in the skeleton plays a key role in the structural integrity of bone. It is still unknown whether bone mineral loss in aging and osteoporosis is solely related to reduction in bone mass, or if it is associated with changes in the elemental concentration in bone tissue (Baslé et al, 1990). Neutron activation analysis provided direct data on the amount of phosphorus, while SPA offered indirect data on both elements, with a preponderance of calcium. The data from the present study on the elemental composition of bone mineral refers to specific anatomic sites of the upper extremities and not to the entire skeleton, *i.e.* PBNAA on the amount of phosphorus in hands (about 5% of the total mineralized bone mass, ICRP, 1975), and SPA on the BMC in a few millimetres thick section of the radius and the ulna (about 0.05% of the total mineralized bone mass). Aloia et al (1987) reported that the BMC at the 8 cm site of the radius showed a higher correlation coefficient with total body calcium than the BMC in the lumbar spine assessed by dual photon absorptiometry (DPA) in both normal women and women with post-menopausal osteoporosis. Furthermore, data given by the same investigators indicate that total body calcium and radial BMC measurements showed the same reliability in the discrimination between normal and osteoporotic women of below 60 years (Aloia et al, 1987). With regard to skeletal phosphorus, there are no available data on the phosphorus in the different skeletal sites. Therefore, extrapolation of the HBP to other skeletal sites of clinical interest, as well as the correlation of HBP with other techniques, such as SPA and DPA, should be considered with caution and must be investigated further.

Controversial results have been reported on the influence of tamoxifen on bone minerals in animal models (Turner et al, 1987; Feldmann et al, 1989; Kalu et al, 1990). Various investigators applying absorptiometric techniques reported that tamoxifen does not lead to osteopenia in humans. Fentiman et al (1989) reported that administration of tamoxifen for up to 6 months does not exert any bone demineralization effect in the lumbar spine and in the femoral neck in pre-menopausal women with mastalgia. Gotfredsen et al (1984), in a longitudinal study, found no difference in radial BMC in breast cancer patients receiving either tamoxifen or placebo for 1 year. However, breast cancer patients in the latter study showed a significant bone loss, which could be attributed to metastatic disease in one fourth of them. In another study, Turken et al (1989) investigated post-menopausal women with breast cancer under tamoxifen treatment. They reported no statistically significant change in bone mineral density in the spine after 12 months of treatment. Wright et al (1990), applying quantitative computed tomography (CT), recently reported normal spinal trabecular density similar in 11 post-menopausal tamoxifen treated breast cancer patients. Love et al (1988) found no statistically

significant difference in bone mineral density in the lumbar spine and the radius between women with breast cancer under tamoxifen treatment for at least 2 years, and patients without treatment. Moreover, they found no relationship between the duration of tamoxifen therapy and bone mineral density. The data given by Love et al (1988) and Turken et al (1989) were adjusted not only for age, weight and height, but also for time since menopause. However, one must be cautious in adjusting the data for time since menopause when trying to identify groups of patients with elevated risk for osteoporosis, because disorders such as breast cancer are frequently related to early menopause.

In the present study all patients had normal lateral radiographs of spine and normal BNI. Therefore, early bone mineral alterations could only be detected by precise and accurate non-traumatic techniques. Phosphorus measurements showed no evidence of osteopenia in the upper extremities in patients treated for at least 1 year with tamoxifen. This finding is in accordance with those obtained by SPA and radiographic morphometry. Other investigators applying absorptiometric techniques that provide indirect data on elemental composition reported similar findings. In addition, no evidence on the influence on the bone mineral status in the upper extremities was found for the duration of tamoxifen treatment, chemotherapy and the presence of non-skeletal metastases. In conclusion, the present study reconfirmed that breast cancer patients treated with tamoxifen are not prone to early osteoporosis.

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References

ALOIA, J. F., VASWANI, A., ATKINS, H., ZANZI, I., ELLIS, K. & COHN, S., 1976. Radiographic morphometry and osteopenia in spinal osteoporosis. *Journal of Nuclear Medicine, 18,* 425–431.

ALOIA, J. F., VASWANI, A., ELLIS, K. & COHN, S., 1987. Bone mineral measurements and the pathogenesis of osteoporosis. In *In Vivo Body Composition Studies.* Ed. by K. J. Ellis, S. Yasumura, W. D. Morgan (The Institute of Physical Sciences, Oxford) pp. 250–260.

BASLE, M. F., MAURAS, Y., AUDRAN, M., CLOCHON, P., REBEL, A. & ALLAIN, P., 1990. Concentration of bone elements in osteoporosis. *Journal of Bone and Mineral Research, 5,* 41–47.

CAMERON, J. & SORENSEN, J., 1963. Measurement of bone mineral *in vivo:* an improved method. *Science, 142,* 230–232.

FELDMANN, S., MINNE, H. W., PAFVIZI, S., PFEIFER, M., LEMPERT, U. G., BAUSS, F. & ZIEGLER, R., 1989. Antiestrogen and antiandrogen administration reduce bone mass in the rat. *Bone and Mineral, 7,* 245–254.

FENTIMAN, I. S., CALEFFI, M., RODIN, A., MURBY, B. & FOGELMAN, I., 1989. Bone mineral content of women receiving tamoxifen for mastalgia. *British Journal of Cancer, 60,* 262–264.

GLAROS, D., XATZIKONSTANTINOU, J., LEODIOU, J. & KALEF-EZRA, J., 1987. A partial-body activation analysis technique for the measurement of phosphorus in bone. In *In Vivo Body Composition Studies.* Ed. by K. J. Ellis, S. Yasumura, W. D. Morgan (The Institute of Physical Sciences, Oxford) pp. 294–299.

GOTFREDSEN, A., CHRISTIANSEN, C. & PALSHOF, T., 1984. The effect of tamoxifen on bone mineral content in premenopausal women with breast cancer. *Cancer, 53,* 853–857.

ICRP, 1975. *Report of the task group on Reference Man,* ICRP Publication 23, (Pergamon Press, Oxford) p. 67.

INGLE, J. N., 1984. Additive hormonal therapy in women with advanced breast cancer. *Cancer, 53,* 766–777.

KALEF-EZRA, J., SIAMOPOULOS, K., KARANTANAS, A., XATZIKONSTANTINOU, J., SFEROPOULOS, G., YASUMURA, S. & GLAROS, D., 1990. Alterations of bone minerals in uremic patients and renal graft recipients. In *Advances in In Vivo Body Composition Analysis.* Ed. by S. Yasumura, J. Harrison, J. K. McNeill, A. Woodhead and A. Dilmanian (Plenum Press, New York) pp. 89–93.

KALU, D. N., SALERNO. E., ECHON, R. M., RAY, M. & HOLIS, B. W., 1990. Effects of estrogen, progesterone and tamoxifen on bone: A comparative study in the ovariectomized rat model. *Journal of Bone and Mineral Research, 5,* S246 (abstract).

LOVE, R. R., MAZESS, R. B., TORMEY, D., BARDEN, H. S., NEWCOMB, P. A. & JORDAN, V. C., 1988. Bone mineral density in women with breast cancer treated with adjuvant tamoxifen for at least two years. *Breast Cancer Research and Treatment, 12,* 297–301.

REINBOLD, W. D., GENANT, H. K., REISER, U. J., REISER, V., HARRIS, S. T. & ETTINGER, B., 1986. Bone mineral content in early postmenopausal and postmenopausal osteoporotic women: comparison of measurement methods. *Radiology, 160,* 469–478.

RIGGS, B. L. & MELTON, L. J., 1986. Involutional osteoporosis. *New England Journal of Medicine, 314,* 1676–1686.

TURKEN, S., SIRIS, E., SELDIN, D., FLASTER, E., HYMAN, G. & LINDSAY, R., 1989. Effects of tamoxifen on spinal bone density in women with breast cancer. *Journal of the National Cancer Institute, 81,* 1086–1088.

TURNER, R. T., WAKLEY, G. K., HANNON, K. S. & BELL, N., 1987. Tamoxifen prevents the skeletal effects of ovarian hormone deficiency in rats. *Journal of Bone and Mineral Research, 2,* 449–456.

WITTLIFF, J. L., 1984. Steroid-hormone receptors in breast cancer. *Cancer, 53,* 589–591.

WRIGHT, C. D. P., EVANS, W. D., MANSEL, R. E. & COMPSTON, J. E., 1990. Effects of long term tamoxifen therapy on bone mas. *Bone, 11,* 221 (abstract).