

ORIGINAL ARTICLE

Hormone therapy and estrogen receptor expression in breast cancer

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Abstract

Postmenopausal hormone therapy (HT) may increase breast cancer risk and influence tumor characteristics. We investigated 321 postmenopausal women aged 50–65 years, with breast cancer, diagnosed and treated at Radiumhemmet, Karolinska Hospital, during 1993–1997. In women using HT ($n = 90$) estrogen receptor concentration (ER) at diagnosis were lower than in non-users ($n = 135$) (1.17 vs 1.70 fmol/ μ g; $p < 0.05$). HT users also had a tendency to less multifocal (5 vs 12%) ($p < 0.05$) and metastatic disease (5% vs 2%) however this was not statistically significant. The estrogen receptor expression is always considered in the judgement on hormone dependency and the clinical decision on adjuvant endocrine therapy. A suppression of ER during HT could tentatively influence the treatment decisions in breast cancer patients and maybe disregard patients from endocrine treatment.

Postmenopausal hormone replacement therapy (HT) with estrogen and progestogen is currently used for relief of vasomotor symptoms and short-term prevention of osteoporosis. Previously it was more widely used at different indications such as long-term prevention of osteoporosis and coronary heart disease [1].

The association between HT and breast cancer risk is incompletely understood. Epidemiological studies have shown an increased risk for breast cancer in current and long-term users [2]. During the last years different studies have reported that combined estrogen/ progestogen treatment may carry a risk for breast cancer beyond that of estrogen alone [3–5]. While HT may increase breast cancer risk there are also some data suggesting that tumors diagnosed in women using HT have a somewhat different biology. Data indicates that they are smaller, have a lower rate of proliferation and a more favorable histology [6,7].

Sex steroid receptor expression is considered a clinically relevant prognostic factor together with axillary node status, tumor size and histologic grade, [8,9]. In general, women with estrogen (ER) and progesterone (PR) receptor positive tumors have

been found to have a better survival [10]. Furthermore the importance of ER and PR positivity as factors to predict responsiveness to endocrine therapy in patients with metastatic breast cancer, as well as in the adjuvant setting, is also well established. Patients lacking ER and/or PR positivity will not be considered for endocrine therapy and higher hormone receptor content has been shown to correlate to a better response [7–9,11].

In clinical practice many different estrogen/progestogen combinations are used for HT. It is well known that the three major therapeutic principles i.e. estrogen alone and estrogen in cyclic or in continuous combination with progestogen have different effects on proliferation in other target organs like the endometrium [12]. At present there is little information about the effects of different treatment regimens on receptor expression the breast epithelium.

In the present study we analyzed data from breast cancer patients with and without ongoing HT at the time of diagnosis. Information on tumor characteristics, reproductive history and previous and current hormonal treatment was obtained from medical records and a self-reported questionnaire.

We compared tumor characteristics and hormone receptor expression between HT users and non-users as well as between different treatment groups.

Materials and methods

During the period 1993–1997 a total of 359 postmenopausal women aged 50–65 years were diagnosed and treated for breast cancer at the Department of Oncology, Radiumhemmet, Karolinska Hospital.

Women were considered postmenopausal according to *one* of the following criteria: absence of menstrual periods for at least 6 months, a FSH value exceeding 25 IU/l, surgical menopause at least 6 months before diagnosis or age ≥ 55 in hysterectomized women. In 33 patients complete medical records were unavailable. Furthermore three hysterectomized women aged < 55 ; one patient using oral contraceptives and one patient with angiosarcoma were excluded. In all 321 postmenopausal patients were included for further analysis.

Information on tumor size, axillary node status, histology, ER and PR status, menopausal status, HT use and parity were obtained from the medical records. The information on menopausal status, HT use, and parity was further confirmed by a questionnaire completed by all women when admitted to the clinic. This instrument covered reproductive history, menstrual data, previous and current disease, previous and current medication including hormonal treatment and height and body weight, and was the primary source of data.

The study was approved by the Ethics committee of the Karolinska Hospital.

Women were defined as HT users if they had been on systemic treatment with estrogen only (estradiol or conjugated estrogens), or estrogen in combination with progestogen (levonorgestrel, norethisterone or medroxyprogesterone acetate) until at least one month before diagnosis, i.e. HT users are "anyone who used HT during diagnosis or had stopped at maximum one month before". The majority of women used estradiol alone or in combination and only 11 women used CEE alone or in combination with MPA. Women using only low potency estrogens, e.g. estriol for treatment of vaginal atrophy were considered as non-users.

ER and PR content were measured on cytosols from tumor homogenates using monoclonal commercial kits, ER-EIA and PR-EIA monoclonal systems according to the instructions of the manufacturer. (Abbott Laboratories, Diagnostics Division, Abbott Park, IL 600064, USA)

In short beads coated with anti-ER/ anti PR (Rat monoclonal) are incubated with specimens (tissue

cytosols/ standards/ control). The tissue has been homogenized on ice with Polytron, centrifuged 20 min 10000 rpm in buffer (10 mM TRIS, 1.5 mM EDTA, 5.0 mM Na_2MoO_4 , the assay is run with standards in duplicate. The receptor content was expressed as fmol/ μg DNA. Values exceeding 0.05 fmol/ μg DNA were considered as ER and PR positive.

In 53 women, because of limited tumor material ER and PR status was assessed by immunohistochemistry (ABBOT ER-ICA, PR-ICA monoclonal system). A standard avidin-biotin-peroxidase immunohistochemical technique was used for immunolocalisation of ER and PR according to the instructions of the manufacturer (Abbott Laboratories, Diagnostics Division, Abbott Park, IL 600064, USA)

A percentage of $> 10\%$ of stained cells was considered as ER/PR positive.

Statistical methods

Differences in means and distributions between HT users and non-users were calculated by the Students t-test for continuous variables and the χ^2 test for categorical variables. Differences between treatment groups were assessed by one-way ANOVA. A p-value < 0.05 was considered statistically significant. A multivariate logistic regression with respect to low/high ER value was performed.

The patients were divided into five equally big groups with respect to ER values. The best discriminating 2-groups constrained combination on HT users/nonusers turned out to be ER = 0–2.67 (low, c. 80% of the patients) and 2.7–9.30 (high, c. 20% of the patients)

Adjustments for age were carried out with linear regression or logistic regression. No adjustment was done when the dependent variable was categorical with more than two categories.

Results

The mean age for the 321 women was 57 years (range 50–65). A total of 128 women were using HT at diagnosis and 193 were non-users (Table I). Current users were on average two years younger than non-users. Parity did not differ and there was no difference in BMI between groups.

Tumor characteristics are shown in Table II. In both groups approximately 75% of the breast cancers were of ductal and 15% of lobular type. Mean tumor size in the two groups was also quite similar, 19 mm for non-users and 20 mm for users respectively. Positive axillary lymph nodes (i.e. axillary nodal metastases) were found in about

Table I. Reproductive characteristics and BMI in HRT users and non-users (mean $\pm$ SD).

	HRT user	HRT nonusers	Unknown	p-value	p adj for age
Age (years)	56 $\pm$ 4.3 n = 128	58 $\pm$ 4.4 n = 193		<0.001	
Time since menopause (months)	82 $\pm$ 58 n = 89	106 $\pm$ 64 n = 166	n = 66	0.004	0.347
BMI	24.7 $\pm$ 3.8 n = 109	25.4 $\pm$ 4.4 n = 160	n = 52	0.2	0.651
Nulliparous /parous	21/105 n = 127	33/159 n = 192	n = 2	0.98	0.552

30% of all women with no difference between groups.

In 80% of the non-users and 79% of the HT users the tumour samples were classified as ER-positive and the corresponding figures for PR positivity were 63% and 70% respectively. While the proportions of ER-positive tumors were similar in both groups women using HT had significantly lower quantitative ER content. The mean value among HT users was 1.17 fmol/ μ g DNA as compared to 1.70 in non-users ($p < 0.05$) ($p = 0.837$ when adjusted for age). However regression analysis on the total material revealed no association between age and ER content. A multivariate logistic regression analysis with respect to low and high ER was performed. Significant factors and age were taken into consideration together with HT. Multiple Odds ratios and 95% confidence intervals were for age 1.01 (0.93–1.11) ($p = 0.77$), PR-positivity 0.36 (0.13–0.97) ($p = 0.043$), HT 0.35 (0.15–0.85) ($p = 0.02$).

PR values did not differ between HT users and non users. In the non-user group there were more women with multifocal breast cancer 5% versus 12% ($p < 0.05$) and numerically more women presented with distant metastases at diagnosis (5% vs 2%) (ns). In the total material more than 80% of the tumors were classified as stage 1 and 2. There were no apparent differences with respect to hormonal treatment at diagnosis.

Women on estrogen only had numerically lower ER and but higher PR content than those treated with estrogen in combination with progestogen (ns) (Table III).

Discussion

Epidemiological studies have clearly demonstrated the importance of sex steroid hormones in the promotion, progression and recurrence of breast cancer. Variations in a woman's hormonal status

Table II. Tumor characteristics in HRT users and non-users expressed as rate (%).

	HRT users	HRT non-users	Unknown	p-value	p adj for age
Histology					
Ductal	87/118 (74%)	130/179 (73%)	n = 24	0.98	
Lobular	18/118 (15%)	28/179 (16%)			
Other	3/118 (3%)	4/179 (2%)			
DCIS	10/118 (8%)	17/179 (9%)			
Tumor size (mm)	20 $\pm$ 15 n = 117	19 $\pm$ 12 n = 177	n = 27	0.56	0.864
Positive nodes					
0	85/119 (71%)	114/168 (68%)	n = 34	0.51	0.206
1–3	26/119 (22%)	36/168 (21%)			
4–	8/119 (7%)	18/168 (11%)			
ER positive	87/110 (79%)	134/168 (80%)	n = 100	0.99	0.959
PR positive	76/108 (70%)	96/153 (63%)	n = 176	0.25	0.729
ER value					
fmol/ μ g DNA	1.17 $\pm$ 1.5 n = 90	1.70 $\pm$ 2.1 n = 135	n = 96	0.045	0.837
PR value					
fmol/ μ g DNA	1.55 $\pm$ 2.4 n = 92	1.6 $\pm$ 2.9 n = 133	n = 96	0.85	0.996
Multifocal tumor	6/121 (5%)	22/177 (12%)	n = 23	0.049	0.852
Metastasis at diagn.	2/127 (2%)	10/192 (5%)	n = 2	0.17	0.145
Stage					
0	10/125 (8%)	17/190 (9%)	n = 5	0.4	0.4
1	62/125 (50%)	85/190 (45%)			
2	48/125 (38%)	77/190 (40%)			
3	3/125 (2%)	2/190 (1%)			
4	2/125 (2%)	10/190 (5%)			

Table III. Tumor characteristics in women on different HRT regimens (mean $\pm$ SD or rate (%)).

	Estrogen only	Continuous combined estrogen/progestogen	Cyclic estrogen/ progestogen	Unknown	p-value	p adj for age
Tumor size mm	23 $\pm$ 18 n = 33	16 $\pm$ 8 n = 47	23 $\pm$ 19 n = 34	14	0.051	0.042
Positive nodes	1.0 $\pm$ 2.2 n = 34	0.6 $\pm$ 1.3 n = 48	1.0 $\pm$ 2.6 n = 34	12	0.64	0.518
0	25/34 (73%)	33/48 (69%)	25/34 (73%)			
1–3	6/34 (18%)	13/48 (27%)	6/34 (18%)			
4	3/34 (9%)	2/48 (4%)	3/34 (9%)			
ER fmol/ μ g DNA	1.1 $\pm$ 1.1 n = 29	1.3 $\pm$ 1.8 n = 36	1.3 $\pm$ 1.6 n = 23	40	0.79	0.795
PR fmol/ μ g DNA	2.3 $\pm$ 3.4 n = 28	1.6 $\pm$ 2.1 n = 38	0.8 $\pm$ 1.4 n = 24	38	0.1	0.108

according to age, phase of the menstrual cycle, menopause and body composition may influence tumor characteristics, the rate of recurrence and survival [13].

In this study we investigated postmenopausal women aged 50–65 years diagnosed and treated for breast cancer at Radiumhemmet during a four year period. We found that women with ongoing HT at diagnosis had a tendency to less metastasis and multifocal disease than those without treatment which is in agreement with a collaborative reanalysis of epidemiological data [2] and different studies showing that HT could be associated with positive tumour characteristics [6,7]. Other studies have pointed out that this could be due to potential biases in patients with HT, e.g. increased medical surveillance or a selective biological effect of hormone use on the growth of less aggressive tumors. Our findings did not support previously reported differences in tumor size, and axillary node status [6,7,14,15].

The diverging results could partly reflect different patient materials and in particular criteria for “current HT use” ranging between 1–12 months prior to diagnosis in different studies. Here information on menopausal status and HT use was collected from each individual woman at the time of presentation thus probably reducing the risk of recall bias.

We found lower ER concentration in breast tumor tissue from HT users compared to non-users. Although there was no difference between tumour samples that were classified as ER-positive in the whole study group when both immunohistochemistry and ELISA were considered, the lower ER concentration in HRT users shows a influence of estrogen that is in line with findings of hormone influence in breast tissue of premenopausal women, where ER expression in normal breast tissue displays a cyclic variation with its lowest values in the luteal phase when both estrogen and progesterone levels are high and proliferation is mostly pronounced

[16]. The same tendency with a cyclic variation has also been shown in premenopausal breast cancer patients and also a negative correlation between ER and circulating estrogen levels was found [17].

The finding of lower ER in postmenopausal HT users diagnosed with breast cancer is in line with some previous data. Bonnier et al. [7] found estrogen receptivity to be both qualitatively and quantitatively lower in HT users. Other studies have shown the opposite. A Danish study and data from Nurses Health study found HT associated with more ER/PR positive tumors than ER/PR negative tumors [18,19]. The explanation could tentatively be different classification of current use, different types of HT used or use of different methods for receptor detection. In USA the most common HT is Conjugated Equine Estrogens with Medroxy Progesterone Acetate as compared to this study where 91% of the women were using estradiol in combination with levonorgestrel or norethisterone. Increased levels of estradiol, the native hormone, would create a hormonal milieu similar to the premenopausal state and a down regulation of the hormone receptor. Receptor expression is rapidly changing by hormonal features. In fact levels are known to vary according to such short time periods as the follicular and menstrual cycle both in normal breast tissue and in breast cancer specimens [16,17]. While breast cancer risk associated with HRT may certainly be duration dependent the effect on hormone receptors would be apparent on short-term.

In clinical practice many different estrogen/progestogen combinations are used for HT. So far there have been some studies on the effects of different treatment regimens on the breast epithelium. In normal breast tissue from both women and surgically postmenopausal macaques combined estrogen/progestogen treatment has formerly been shown to induce more proliferation than treatment with estrogen alone [20,21]. However in this study we did not

find any statistical differences between the different treatment groups, although numbers are small. There was a tendency to estrogen only users having lower ER, but higher PR content.

Here we have shown a lower ER expression in tumors from women using HT compared to non users. In conclusion the present data add to the growing notion that ongoing HT at the time of breast cancer diagnosis may influence certain tumor characteristics such as expression of steroid receptors. This exploratory study shall be interpreted with caution and the results from it are suggestive and need confirmation in other studies.

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