

Endogenous Estrogens and Risk of Breast Cancer by Estrogen Receptor Status: A Prospective Study in Postmenopausal Women¹

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Abstract

A positive association between postmenopausal serum levels of total estradiol, percentage of free estradiol, and percentage of estradiol not bound to sex hormone-binding globulin (SHBG) and breast cancer risk was recently reported by the New York University Women's Health Study (P. Toniolo *et al.*, *J. Natl. Cancer Inst.*, 87: 190–197, 1995). Data from this prospective study are used to assess whether the observed associations differ according to estrogen receptor (ER) status of the tumor. Between 1985 and 1991, 7063 postmenopausal women donated blood and completed questionnaires at a large breast cancer screening clinic in New York City. Before 1991, 130 cases of first primary breast cancer were identified by active follow-up of the cohort. For each case, two controls were selected, matching the case on age at first blood donation and length of storage of specimens. Biochemical analyses were performed on sera that had been stored at -80°C since sampling. ER information was abstracted from pathology reports. Separate statistical analyses were conducted for ER-positive, ER-negative, and ER-unknown groups (53, 23, and 54 matched sets, respectively). In each of the 3 groups, the mean estradiol and the mean percentage of free estradiol were greater (21–28% and 6–7%, respectively) in cases than in controls. Conversely, the mean percentage of estradiol bound to SHBG was 9–12% lower in cases than in controls. The logistic regression coefficients measuring the strength of the association between estradiol and its free and SHBG-bound fractions and breast cancer risk were similar in the ER-positive, ER-negative, and ER-unknown groups. These data suggest that in postmenopausal women, the association of endogenous estrogens with breast cancer risk is independent of the ER status of the tumor. This result is more compatible with the hypothesis of a progression from ER-positive to ER-negative tumors than with the

hypothesis that ER status identifies two distinct types of breast cancer.

Introduction

Whereas ERs³ have a well-established role in assessing the prognosis of breast cancer and predicting response to hormonal therapy, their role in the etiology of the disease is not clear. Using a two-stage model for carcinogenesis, Moolgavkar *et al.* (1) proposed that ER+ and ER– tumors represent different stages in the disease process, and that “malignant tumors are initially ER+ and lose their hormone dependence through clonal evolution.” However, others have proposed that ER status identifies different types of breast cancer (2–4).

Estrogens are involved in the growth and differentiation of normal breast tissue and are thought to play an important role in the development and progression of breast cancer (5). The NYU Women's Health Study recently reported a positive association of serum levels of total estradiol and percentage of free estradiol with subsequent risk of breast cancer in postmenopausal women (6). The percentage of estradiol bound to SHBG was negatively associated with breast cancer risk, a result consistent with the hypothesis that this fraction is not available to the target cells.

ERs bind to estradiol with high specificity and affinity. Consequences of the estradiol-ER interaction include DNA synthesis, cell division, and production of biologically active proteins such as growth factors and progesterone receptor proteins (5, 7). If ER status distinguishes different types of tumors, one would expect the ER status at diagnosis to reflect the receptor status of the tumor cells of origin. If, in addition, ERs must be present for estrogens to stimulate cancer cell growth, then estrogens would be expected to be a risk factor for ER+ tumors but not ER– tumors. The NYU Women's Health Study is the first prospective study to offer the opportunity to examine whether the increased risk of breast cancer associated with higher postmenopausal levels of bioavailable estrogens varies according to ER status.

Materials and Methods

Between March 1985 and June 1991, the NYU Women's Health Study enrolled a cohort of 14,291 women (of whom, 49.7% were postmenopausal) at a large breast cancer screening center in New York City (6, 8). At the time of enrollment, women were asked to donate blood and complete a self-administered questionnaire. Serum was frozen at -80°C for future biochemical assays. Women who had taken hormonal medications in the 6 months preceding their visit were not eligible.

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³ The abbreviations used are: ER, estrogen receptor; ER+, ER positive; ER–, ER negative; NYU, New York University; SHBG, sex hormone-binding globulin.

Cases of breast carcinoma were identified primarily through active follow-up of the cohort (8). Cases were confirmed by reviewing clinical and pathology records from hospitals. One hundred thirty cases were diagnosed before December 31, 1990, among the 7063 cohort members who were postmenopausal at enrollment (6). For each case, two controls were selected at random from the risk set of cohort members who were alive and free of breast cancer when the case was diagnosed, and who matched the case on age at enrollment, menopausal status, and approximate date of blood donation. Of the 260 controls initially selected, 12 were excluded (6 had been taking corticosteroids at the time of blood donation, 3 had biochemical assays done on a different day than the case, and 3 had serum follicle-stimulating hormone levels not consistent with postmenopausal status). As a result, 130 cases and 248 controls are included in this analysis.

Biochemical methods have been described in a previous publication (6). Total estradiol levels were measured by RIA kits (Pantex, Santa Monica, CA) without prior chromatographic separation. ER information was abstracted from the laboratory and pathology reports of each case. Cutpoints provided by the laboratory that performed the assay were used to classify tumors as ER+ or ER-.

Statistical Methods. Estradiol concentrations were \log_e transformed to reduce departures from normality. Within each group (ER+, ER-, ER-unknown), the paired *t* test was used to compare hormone levels of the cases to the mean hormone levels of their matched controls.

Conditional logistic regression was used to account for the case-control matching (9). Separate analyses were conducted for ER+, ER-, and ER-unknown groups, as well as a combined analysis to formally test for differences between the groups. Hormone measurements were analyzed as continuous variables because of the small sample sizes. Likelihood ratio tests were used to assess statistical significance. Conditional logistic regression coefficients are reported unadjusted and adjusted for Quetelet's index [weight (kg) divided by height (m^2)] because initial analyses (6) showed that only weight or Quetelet's index altered the regression coefficients of the hormonal variables by at least 10%. All reported *P* values are 2-sided.

Results

ER status was available for 76 (58%) of the 130 cases: 53 (70%) had ER+ tumors and 23 (30%) had ER- tumors. Women in the ER- group were slightly older at blood donation (median age, 60.7 years) than women in the ER+ and ER unknown groups (median age, 59.2 years). The median age at diagnosis was similar in the three groups (61.2 years). The large majority of women (86%) were Caucasian. There were no differences with respect to body weight (Quetelet's index) between the 3 groups: overall, the median Quetelet's index was 25.6 kg/m^2 .

Table 1 shows the pathological characteristics of the tumors. Most of the tumors were ductal carcinomas. There was a larger proportion of stage III and IV carcinomas in the ER- group (23%) than in the ER+ group (4%, *P* = 0.02, Fisher's exact test). There was no statistically significant difference in grade of differentiation between the 3 groups, but these data were missing for more than one-half of the tumors.

Table 2 reports the estrogen measurements in the 3 groups of cases and their matched controls. Within each of the 3 groups, controls had lower values of total estradiol and percentage of free estradiol than cases, and larger values of per-

Table 1 Pathological characteristics of the tumors by ER status

	Tumor ER status		
	ER+ (n = 53)	ER- (n = 23)	ER-unknown (n = 54)
Ductal carcinoma (%)	79	87	76
Stage at diagnosis (%)			
<i>In situ</i>	0	4	17
I/II	96	73	83
III/IV	4	23	0
Grade of differentiation (%)			
Well	11	17	5
Moderately	28	9	11
Poorly	13	13	15
Unknown	47	61	69

centage of estradiol bound to SHBG. The differences observed between cases and controls were similar in the 3 groups: the mean estradiol was 21–28% greater and the mean percentage of free estradiol was 6–7% greater in cases than in controls. Conversely, the mean percentage of estradiol bound to SHBG was 9–12% lower in cases than in controls.

For all 3 groups (ER+, ER-, and ER-unknown), Table 3 reports the logistic regression coefficients measuring the strength of the association of risk of breast cancer with Quetelet's index and hormone measurements. There was a nonsignificant positive association between Quetelet's index and risk of breast cancer in each of the 3 groups. In unadjusted analyses, there was a positive association between total estradiol and risk of breast cancer, with similar regression coefficients in the 3 groups. The percentage of free estradiol was positively associated with risk of breast cancer, whereas the percentage of estradiol bound to SHBG had a protective effect in each ER group. For each of the 3 hormonal assays, and within each ER group, adjusting for Quetelet's index resulted in a reduction of the magnitude of the regression coefficients. Overall, although the statistical significance varied from one group to the other, which is at least partly due to the different sizes of the groups, the regression coefficients measuring the associations between hormone measurements and risk of breast cancer were similar in the ER+, ER-, and ER-unknown groups.

In an analysis including all ER groups, the addition of indicator variables for ER status and interaction terms between these indicator variables and the hormonal measurements, to models containing the hormonal measurements only, allowed us to test whether the regression coefficients of the hormone measurements varied significantly with ER status (9). None of these interaction tests was significant. Thus, there was no statistical evidence of a differential effect of estradiol or its unbound or SHBG-bound fractions on breast cancer risk in the 3 groups.

Discussion

We reported previously a positive association, irrespective of ER status, between bioavailable estrogens and subsequent risk of breast cancer in postmenopausal women (6). The main result of the present analysis is that the observed associations appear to be independent of the ER status of the tumor.

This result, if replicated in studies conducted in different populations and with larger sample sizes, would favor the hypothesis of a progression from ER+ to ER- tumors. In support of this hypothesis, studies have shown that ER-

Table 2 Mean and range of serum levels of total estradiol, percentage of estradiol free, and percentage of estradiol bound to SHBG by tumor ER status

	Tumor ER status					
	ER +		ER -		ER-unknown	
	Cases (n = 53)	Controls (n = 101)	Cases (n = 23)	Controls (n = 45)	Cases (n = 54)	Controls (n = 102)
Estradiol (pg/ml)						
Mean ^a	36.2	29.2	29.9	23.4	32.8	27.0
Range	10-106	3-118	10-71	4-79	10-78	5-145
P value ^b		0.05		0.07		0.04
Free estradiol (%)						
Mean	1.41	1.33	1.39	1.30	1.44	1.36
Range	0.8-2.1	0.8-2.0	0.9-2.1	0.7-2.3	0.8-2.2	0.6-2.4
P value ^b		0.05		0.08		0.03
SHBG-bound estradiol (%)						
Mean	38.9	42.9	40.4	45.9	40.2	45.7
Range	10.7-60.4	17.1-66.3	22.3-57.5	22.8-67.9	20.9-62.3	16.7-68.1
P value ^b		0.03		0.02		0.001

^a Geometric mean.^b Determined by paired *t* test.

Table 3 Conditional logistic regression coefficients for breast cancer associated with serum levels of estradiol, percentage of estradiol free, and percentage of estradiol bound to SHBG by tumor ER status

	Tumor ER status					
	ER +		ER -		ER unknown	
	Crude	Adjusted ^a	Crude	Adjusted ^a	Crude	Adjusted ^a
Quetelet's index (ln kg/m ²)						
β	1.947		1.843		1.767	
SE (β)	1.07		1.61		1.15	
P value	0.07		0.24		0.12	
Estradiol (ln pg/ml)						
β	0.789	0.651	0.788	0.680	0.629	0.515
SE (β)	0.371	0.377	0.492	0.536	0.329	0.349
P value	0.02	0.07	0.09	0.19	0.05	0.13
Free estradiol (%)						
β	1.600	1.204	2.267	2.224	1.613	1.398
SE (β)	0.776	0.922	1.29	1.57	0.819	0.862
P value	0.04	0.20	0.06	0.14	0.04	0.10
SHBG-bound estradiol (%)						
β	-0.032	-0.024	-0.064	-0.069	-0.058	-0.056
SE (β)	0.016	0.019	0.030	0.036	0.020	0.021
P value	0.03	0.21	0.02	0.05	0.001	0.004

^a For Quetelet's index (ln kg/m²).

tumors are less differentiated than ER+ tumors (5). In addition, although most studies found no association between stage at diagnosis and ER status (5), a recent study found that tumor size was negatively associated with ER positivity in breast cancers detected by screening (10). Our data similarly showed significantly more stage III and IV tumors in the ER- group (23%) than in the ER+ group (4%; *P* = 0.02). We did not observe differences in grade of differentiation between the three groups. However, data on differentiation was missing for more than one-half of the tumors, limiting our ability to look at this factor.

Our results are in agreement with epidemiological studies comparing risk factor profiles by ER status. A recent review of these studies (5) concluded that there was an overlap for most risk factors (with the exception of age, race, and nulliparity), supporting the notion that ER status may represent different stages in the same disease process. A number of studies have compared blood levels of estrogens in ER+ and ER- breast

cancer patients and reported mixed results (11-14). However, these studies are not very informative because measurements taken after diagnosis could be influenced by the presence of a clinically detectable tumor.

One limitation of our study is the relatively large proportion of cases (42%) for whom ER information was not available. For eight cases who presented with *in situ* tumors, this was due to lack of tumor tissue available for the assay. For other cases, the pathology records that were sent to us did not include the results of the ER assay, although in 56% of those, there was evidence that tissue had been submitted for the assay. In any event, the case-control differences in estrogen levels that we observed were of similar magnitude in the 3 groups. If estrogens had a different effect on ER+ and ER- tumors, one would expect that results would differ between these two groups, whereas results in the ER-unknown group would be intermediate.

Misclassification is another possible limitation of our study because ER assays were done with different methods in different laboratories. However, because studies have shown excellent agreement between methods (15–17), we expect that misclassification of ER status had little impact on our results.

Total serum estradiol levels were lower, for both cases and controls, in the ER– group than in the ER+ group, whereas values in the ER-unknown group were intermediate. This result was unexpected, in particular among controls. In additional analyses (data not shown), we found that age at blood donation partially explained these differences because serum estradiol levels decreased with increasing age, and women in the ER– group were slightly older at blood donation than women in the 2 other groups. In any case, levels of both percentage of free estradiol and percentage of estradiol bound to SHBG were comparable in the 3 control groups.

In conclusion, a positive association of serum total estradiol and its free and non-SHBG-bound fractions was observed with risk of both ER+ and ER– breast cancer in postmenopausal women. These data favor the hypothesis of a progression from ER+ to ER– tumors rather than the hypothesis that ER status identifies different tumor types. However, a better understanding of the mechanisms by which estrogens increase the risk of breast cancer is necessary to further interpret these data. Finally, it has been proposed (18) that characterization of breast tumors on both progesterone receptors and ERs is more relevant in defining different types of tumors than characterization on ERs only. We were unable to perform such an analysis because of the limited number of cases to date in our study. However, with increasing follow-up, such an analysis will be possible in the future.

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