Urinary estrogen metabolites in women at high risk for breast cancer

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Objective: This study explored whether average urinary estrogen metabolites in breast cancer high-risk women can be used to identify a subgroup of women at particularly high risk to develop breast cancer, to which prevention strategies should be addressed.

Methods: The population consisted of 77 high-risk women, 30 breast cancer patients and 41 controls. All subjects answered a standardized questionnaire; height and weight and spot urine samples were also obtained. Urine hydroxysterogen metabolites were measured in triplicate by enzyme immunoassay, and the estrogen metabolite ratios for each individual were calculated. Results: The 2:16 OHE ratio (2-hydroxyestrone/16-alpha-hydroxyestrone) in women at high risk for breast cancer was similar to that observed in the breast cancer group (1.76 ± 2.33 versus 1.29 ± 0.80) and lower than in controls (2.47 ± 1.14; P = 0.00). At the multivariate linear regression model, the 2:16 OHE ratio was significantly associated with diagnosis (P = 0.000 for both the high risk and breast cancer group versus the controls) and body mass index (P = 0.005), but not with age (P = 0.604), or smoking history (P = 0.478). Conclusions: This study suggests that lower urinary 2:16 OHE ratios are predictors of breast cancer risk. Profiling estrogen metabolites may identify women who are more probably to develop breast cancer within a population of women with known risk factors and may help to further elucidate the clinical relevance of urinary 2:16 OHE ratios as clinical markers and prognostic indicators in this population.

Introduction

Breast cancer is a major public health concern because of its heavy contribution to both morbidity and mortality in the USA and in the world. Several risk factors have been associated with breast cancer, most of which reflect the cumulative estrogen exposure during a woman’s lifetime (1). This has initiated a large body of research on the role of estrogen and estrogen metabolites as both cancer initiators and promoters.

Estrogen metabolites have been examined in several breast cancer studies, particularly 16-alpha-hydroxyestrone (16α-OHE1) and 2-hydroxyestrone. The estrogenic properties of these two compounds vary according to their ability to bind to the estrogen receptor. The 16α-OHE1 metabolite is an estrogen-like product through its binding to the estrogen receptor; in contrast, the 2-hydroxyestrone metabolite has low or no estrogen activity because of its low affinity with the estrogen receptor. The production of the two metabolites is mutually exclusive; therefore, any external factor modifying the production of one of the two compounds is also responsible for the indirect modification of the other. For example, a diet rich in cruciferous vegetables may favorably increase the 2:16 OHE ratio (2).

Abbreviations: BMI, body mass index; 2:16 OHE, 2-hydroxyestrone:16-alpha-hydroxyestrone; 16α-OHE1, 16-alpha-hydroxyestrone.

To date, the studies that have evaluated the relationship between estrogen metabolite levels and breast cancer (3–14) have obtained inconsistent results. Earlier studies (3,4) first measured the role of 16α-OHE1 using a radiometric assay in a small sample of women with breast and endometrial cancer and suggested elevated levels of 16α-OHE1 among women with breast and endometrial cancer as compared with the controls. Since then, at least 11 studies have evaluated the association between estrogen metabolites in both urine and serum and breast cancer, including six case–control, four nested case–control and one case–cohort study (5–15). The results are mixed results but tend to support an association between estrogen metabolism and breast cancer.

Though the role of estrogen metabolites has been studied in breast cancer patients, estrogen metabolism in patients who are at high risk for developing breast cancer has not been clarified as yet. Most recently, one study compared urinary estrogen metabolites, conjugates and deurinating DNA adducts between 12 high-risk women, 17 women with breast cancer and 46 control women and found a significant difference in the ratio of these compounds in breast cancer and high-risk women compared with controls; however, the sample size was small (15).

The present study was designed to measure estrogen metabolism in a large group of high-risk women and assess if this urinary biomarker can be a predictor of future breast cancer development. In addition, the study addresses whether the urinary 2:16 OHE ratio may be linked to specific epidemiologic risk factors. Understanding the relationship between metabolites, environmental and dietary factors interacting with estrogen metabolites production or elimination may help elucidate the underlying mechanisms of breast cancer as well as identify potential measures of prevention.

Methods

Women were recruited from the High Risk Breast Cancer Program at Magee-Women’s Hospital, a program where women who are at high risk of developing breast cancer are referred for periodic screening and prevention. Risk factors for these women included first-degree family history, lobular carcinoma in situ, ductal carcinoma in situ, fibrocytic breast disease, mutations in either BRCA1 or BRCA2 and Ashkenazi Jewish descent. All consecutive patients coming to the high-risk clinic from June 2007 through December 2007 were enrolled (participation rate 100%). Four of the urine samples were not preserved with addition of ascorbic acid and thus, the corresponding women were excluded (4.9% of the total sample).

Breast cancer patients were identified through the Breast Cancer Surgical Registry at Magee-Women’s Hospital, a database in existence since 2005, which includes all consecutive women undergoing surgery for breast cancer at Magee. At the time of surgery, women sign an informed consent to be included in the registry so that clinical annotation is collected from their charts at the time of surgery; breast tissue and a urine sample are stored in the pathology repository and linked to the clinical annotation electronic file. Data from all women included in the surgical registry between June and December 2007 were utilized for the present study. However, two women had to be excluded because urine was collected postsurgery (6%), one urine sample was not preserved with the addition of ascorbic acid and was also excluded (3%).

Control women were a convenience sample of volunteers recruited as part of a study on hormones, bone density and genetic markers of susceptibility (16). Recruitment of healthy subjects occurred between 2002 and 2006; the response rate was 93% (41 of 44).

Women were asked to answer questions from a standardized survey, which was administered by one research assistant. From these surveys, data regarding family history of cancer, smoking history and alcohol use were obtained. Alcohol use data were not available for the control group. Patients’ height and weight were also obtained at the time of interview in order to calculate body mass index (BMI). Menopausal status was defined as lack of menstrual periods for 6 months before the interview. In women where menopausal status was not known, a combination of age (≥55 years) and presence of menopausal symptoms was used as cutoff for the definition of postmenopausal status.

Spot urine samples were then obtained at the time of visit and presurgery/pretherapy in breast cancer women and were sent to a tissue bank for storage. None of the subjects received estrogen-containing treatment for at least 3 months prior to urine sample collection.
Estrogen metabolites were determined in urine collected with a standardized procedure, preserved by addition of ascorbic acid 400 mg and frozen at −20°C after collection. The C-2 and C-16 hydroxysteroid metabolites were measured in triplicate by enzyme immunoassay (enzyme-linked immunosorbent assay), using kits from Immuna Care (Bethlehem, PA), and the estrogen metabolite ratios for each individual calculated from these values. Repeated measurements were obtained from random samples to ensure reliability of results. The coefficient of variation for the metabolite assays was 0.033, with a range of 0.0044–0.082. A small reproducibility study was nested within the main study. Five healthy women collected urine samples in two different days, and the samples were blinded, coded and tested for assessing intra- and intersubject variability.

The 2:16 OHE ratio is reported to be reproducible during the day, during the menstrual cycle and within 6 months interval in postmenopausal women (17–19). The kit utilized for the urine measurements does not require adjustment for creatinine; however, the statistical analyses were conducted on both creatinine-adjusted and unadjusted data for confirmatory purposes.

Informed consent was obtained from each patient prior to survey administration and sample collection. Study procedures were approved by the University of Pittsburgh Medical Center Institutional Review Board.

Statistical analysis

The one-way analysis of variance was used to test differences between mean age for the three subgroups. The 2:16 OHE ratios and BMI were not normally distributed; therefore, medians were calculated for each subgroup. A comparison between all three groups was performed using the Kruskal–Wallis equality-of-populations rank test for multiple comparisons. The Wilcoxon rank-sum (Mann–Whitney) test was used to compare medians between the high-risk group and controls and between the breast cancer group and controls. Chi-square exact test was used to compare proportions. In addition, the association of 2:16 OHE ratios with BMI, smoking history and alcohol history and age was assessed in a multivariate linear regression model. Partial correlation coefficients were calculated to measure the strength of the linear relationship between 2:16 OHE ratios and BMI, smoking history and alcohol history and age was assessed in a multivariate linear regression model. Partial correlation coefficients were calculated to measure the strength of the linear relationship between 2:16 OHE ratios and diagnosis after controlling for the effects of age, smoking and BMI. In the high-risk group alone, the associations between 2:16 OHE ratios and BMI, smoking history and alcohol history and age was assessed in a multivariate linear regression model. Partial correlation coefficients were calculated to measure the strength of the linear relationship between 2:16 OHE ratios and diagnosis after controlling for the effects of age, smoking and BMI.

Results

There were 77 high-risk patients, 30 breast cancer patients and 41 controls. Characteristics of the study groups are shown in Table I and Table II. Of the 77 high-risk patients, 21 had a diagnosis of carcinoma in situ (11 lobular carcinoma in situ and 10 ductal carcinoma in situ; Table I). The mean age was lower in the controls than in breast cancer and high-risk women, and the difference in age among all groups was found to be statistically significant (P = 0.0001). All groups comprised mostly Caucasian women: 76 of 77 in the high-risk group, all the women in the breast cancer group and 40 of 41 in the control group. More women in the high-risk group were postmenopausal than that in the breast cancer or in the control group, although the difference was not statistically significant. The available reproducible factors (age at menarche and being ever pregnant) did not significantly differ across groups.

The 2:16 OHE ratio differed significantly among groups (P = 0.0001). The 2:16 OHE ratio was significantly lower in the high-risk group compared with controls (P = 0.00). Similarly, the 2:16 OHE ratio was significantly lower in the breast cancer group compared with controls (P = 0.00). There was no significant difference between the ratios of the high-risk group and the breast cancer group. The median values of 2:16 OHE ratio in postmenopausal controls was 2.13, in breast cancer women was 1.15 and in high-risk women was 0.97.

When the patients with carcinoma in situ were separated in the analysis, the results were similar: the median 2:16 OHE ratio in the high-risk group was 1.16, significantly lower compared with controls (P = 0.035). Within the high-risk group, the presence of a family history of a first-degree relative with breast or ovarian cancer did not affect the 2:16 OHE ratio (Figure 1).

BMI differed among groups (P = 0.002). Both the high-risk group and the breast cancer group had significantly higher BMI than the control group (P = 0.0016 and P = 0.004, respectively). The 2:16 OHE ratio was significantly associated with BMI when evaluating all (Figure 2) study subjects together (P = 0.0006). However, when analysis was performed within each group, there was no significant association between 2:16 OHE ratio and BMI in the high risk and breast cancer groups. There was a significant association between 2:16 OHE ratio and BMI in the control group (P = 0.045).

There was a significant association between 2:16 OHE ratio and alcohol use in the groups where the information was available (high risk and breast cancer) (P = 0.02). There was no association between 2:16 OHE ratio and smoking history among all three groups or within each group. Likewise, there was no association between 2:16 OHE ratio and age among all three groups.

### Table I. Distribution of risk factors in the high-risk group (N = 77)

<table>
<thead>
<tr>
<th>Family history</th>
<th>Lobular carcinoma in situ</th>
<th>Ductal carcinoma in situ</th>
<th>Atypia</th>
<th>Fibrocytic breast disease</th>
<th>BRCA1/2</th>
<th>Ashkenazi Jewish</th>
<th>Benign breast disease</th>
<th>Ovarian cancer</th>
<th>PASH*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients in each high-risk group (%)</td>
<td>31 (40.3)</td>
<td>11 (14.3)</td>
<td>10 (13.0)</td>
<td>10 (13.0)</td>
<td>7 (9.1)</td>
<td>1 (1.3)</td>
<td>3 (9.9)</td>
<td>3 (9.9)</td>
<td></td>
</tr>
</tbody>
</table>

*Data missing for one subject.

**Forty subjects are counted more than once because of multiple risk factors; 37 patients had only one risk factor.

### Table II. Subjects characteristics according to study group

<table>
<thead>
<tr>
<th>Age (years) mean ± SD</th>
<th>2 OHE (ng/ml), median</th>
<th>16 OHE (ng/ml), median</th>
<th>2:16 OHE ratio, median</th>
<th>Postmenopausal, n (%)</th>
<th>BMI (kg/m²), mean ± SD</th>
<th>Regular alcohol use, n (%)</th>
<th>Ever smokers, n (%)</th>
<th>First-degree breast cancer, n (%)</th>
<th>Never pregnant, n (%)</th>
<th>Age at menarche, median (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High risk (N = 77)*</td>
<td>54 ± 9.5</td>
<td>17.72</td>
<td>14.04</td>
<td>1.15</td>
<td>35 (45.4)</td>
<td>26.62 (27.1 ± 5.45)</td>
<td>28 (37.3)*</td>
<td>25 (32.9)*</td>
<td>24</td>
<td>9 (11.7)</td>
</tr>
<tr>
<td>Breast cancer (N = 30)</td>
<td>57 ± 9.8</td>
<td>2.44</td>
<td>2.29</td>
<td>1.09</td>
<td>12 (40)</td>
<td>26.63 (29.0 ± 7.72)</td>
<td>10 (34.5)*</td>
<td>2 (6.7)</td>
<td>N/A</td>
<td>6 (20)</td>
</tr>
<tr>
<td>Control (N = 41)</td>
<td>47 ± 10.3</td>
<td>13.98</td>
<td>5.90</td>
<td>2.22</td>
<td>13 (32)</td>
<td>23.05 (24.0 ± 4.24)</td>
<td>N/A</td>
<td>7 (17.1)</td>
<td>N/A</td>
<td>8 (19)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0001*</td>
<td>0.0001</td>
<td>0.0001</td>
<td>n.s.*</td>
<td>0.002</td>
<td>N/A</td>
<td>n.s.*</td>
<td>N/A</td>
<td>n.s.*</td>
<td>n.s.*</td>
</tr>
</tbody>
</table>

*Data missing for one subject.

*P-value missing for two subjects.

**P-value: comparison of medians across groups, Kruskal–Wallis equality-of-populations rank test.

**P-value: one-way analysis of variance, between means comparison.

*Chi-square exact test; n.s., not statistically significant.
At the multivariate linear regression model, the 2:16 OHE ratio was independently and significantly associated with diagnosis (Table III); similarly, BMI [partial correlation coefficient \((r = -0.2434, P = 0.005)\), but not age \((r = 0.0452, P = 0.604)\), or smoking history \((r = -0.0618, P = 0.478)\)] was associated with estrogen metabolites. Alcohol was not analyzed given only two of the three groups had available data.

### Discussion

Our results support the hypothesis that preferential 16-alpha-hydroxylation of estrogen metabolism is already present in women at increased risk of breast cancer. In our study, women with a known increased risk of developing breast cancer were found to have a lower 2:16 OHE metabolite ratio in their urine compared with healthy women, and these ratios were similar to breast cancer patients as well. The result held even after adjustment for possible confounding factors in the multivariate analysis, indicating that the metabolite ratio is an independent predictor of breast cancer risk. There is already some evidence in the literature that patients diagnosed with breast cancer have increased 16-alpha-hydroxylation (2–13) but no convincing data on the role of the 2:16 OHE ratio in identifying high-risk breast cancer women as a separate group from otherwise healthy women.

In addition, our results showed that 2:16 OHE ratios were significantly associated with known risk factors such as BMI and alcohol use. This study lends evidence to support the idea that variations in estrogen metabolism can confer risk and are associated with known risk factors for developing breast cancer. Obesity is associated with increased breast cancer risk in postmenopausal women (20,21). In addition, obesity has also been linked to preferential estrogen metabolism via the 16-alpha-hydroxylation pathway; thus, a prediction of the mechanism by which obesity could increase breast cancer risk would be through a lowering of the 2:16 ratio in favor of the 16 pathway (23). In this study, BMI was significantly associated with the 2:16 OHE ratio overall among all three groups, where increased BMI was associated with a lower 2:16 OHE ratio. Similar results were reported by other investigators in healthy women (20). However, the association seems specifically restricted to the control group when analyzed separately. Although a potential explanation for this finding is that the subgroup analysis did not include a large enough number of subjects within each group, it is also possible that the physiologic effect of body mass on estrogen metabolism is visible in healthy women, but disappears when breast cancer is diagnosed, and is not measurable when other concurrent risk factors are present, as observed in high-risk women. This hypothesis requires further investigation.

Our data show a significant association between alcohol use, defined as at least one drink per day or an average of seven per week, and 2:16 OHE ratio. An alcohol-induced rise in estrogens as a consequence of alcohol catabolism in the liver has been reported (23). This could be the biological basis for the observed association. The only study that looked at the association between alcohol and wine consumption in healthy women did not report a clear association (20). Since alcohol intake is a modifiable risk factor, the understanding of its relationship with 2:16 OHE ratio may be a first step to the potential use of the metabolite ratio as both a marker of cancer risk and of the effectiveness of changes in behavioral risk factors.

Epidemiological studies have not been able to consistently show a clear relationship between cigarette smoking and risk of developing breast cancer; the results of the present analysis show no association between smoking and 2:16 OHE ratio. This is in contrast with what was expected since smoking has been reported to increase induction of the 2-hydroxylation metabolic pathway (24). However, the few epidemiological studies conducted on healthy women showed no difference in estrogen metabolites with smoking status (22) or smoking dose (20), in line with our findings.

Family history of a first-degree family member with breast cancer confers a 2- to 4-fold risk of developing breast cancer (25,26). Outside of genetic risk with BRCA1 and BRCA2, it is estimated that 16% of breast cancers are due to unidentified hereditary factors. In our study, the average 2:16 OHE ratio of women with a positive first-degree family history of cancer did not differ from the overall ratio of the high-risk group. This result could be attributed to the fact that family history is a product of both the genetic makeup and environmental factors. Estrogen metabolism occurs through enzymes whose activity is determined by the presence of specific genetic polymorphisms, thus it can be defined as unique to each individual. However, the metabolism is also influenced by a number of environmental factors, which change over a lifetime; thus, the 2:16 OHE ratio may not be the appropriate biomarker to capture the results of such complex interaction.

A limitation of this study is the convenience sample of controls used for comparison; further studies using an age matched control group would help to clarify this result. Another limitation is the
collection of a unique sample of urine spot. This one-time measurement may not adequately reflect the historical variations of the 2:16 OHE ratio, but it only gives a snap shot of the current estrogen metabolism. In addition, reliable data on menopausal status were not available for all the subjects. However, repeated analysis of the data according to the available information on menopausal status, as well as sensitivity analysis using different age cutoffs, did not substantially change the findings. A complete reproductive history was also missing for a substantial part of the population, thus preventing us from adjusting the analysis for such factors. These limitations require caution in the interpretation of the findings.

However, our research includes the largest population of high-risk women in which clinical, epidemiological and estrogen metabolites information were available and suggests a significantly lower 2:16 OHE ratio in women who have known breast cancer risk factors compared with healthy women. There was an additional significant association specifically with BMI and alcohol use, which also supports the evidence that these factors affect estrogen metabolism. Profiling estrogen metabolites may identify women who are more likely to develop breast cancer within a population of women with known risk factors. This relationship may help to further elucidate the clinical relevance of using urinary 2:16 OHE ratios as additional clinical markers and prognostic indicators in this population. Moreover, given that estrogen metabolism is one of few modifiable risk factors for developing breast cancer, knowledge of the factors that affect estrogen metabolism itself may lend support to or even uncover new recommendations about risk reduction strategies.

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**References**


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