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.
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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 International 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 the breast cancer group. 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).

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 (3.9)</td>
<td>11 (14.3)</td>
<td>1 (1.3)</td>
</tr>
</tbody>
</table>

aDuctal pseudoangiomatous stromal hyperplasia.
bForty 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), median</th>
<th>Regular alcohol use, n (%)</th>
<th>Ever smokers, n (%)</th>
<th>First-degree breast cancer history, n (%)</th>
<th>Never pregnant, n (%)</th>
<th>Age at menarche, median (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High risk (N = 77b)</td>
<td>54 ± 9.5</td>
<td>17.72</td>
<td>14.04</td>
<td>1.15 (1.76 ± 2.33)</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>2.24</td>
<td>2.29</td>
<td>1.09 (1.29 ± 0.80)</td>
<td>12 (40)</td>
<td>26.63 (29.0 ± 7.72)</td>
<td>10 (34.5)</td>
<td>7 (26.7)</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 (2.47 ± 1.14)</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>
</tbody>
</table>

| P-value | 0.0001c                    | 0.0001c | 0.0001c | n.s.      | 0.002c | n.s. | 0.008c | n.s. | n.s. |

cData missing for one subject.

dData 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.

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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 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

![Fig. 1. The 2:16 OHE ratio in the study group.](image1)

<table>
<thead>
<tr>
<th>Table III. Adjusted correlation coefficients* between 2:16 OHE and clinical diagnosis</th>
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<tbody>
<tr>
<td>&lt;br&gt; n = 138</td>
</tr>
<tr>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>r (P)</td>
</tr>
<tr>
<td>2:16 OHE reference</td>
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</tbody>
</table>

n, number of subjects included in the analysis. R, partial correlation coefficient. <br> *Adjusted for age, BMI and smoking history and menopausal status.

![Fig. 2. Linear correlation coefficient between 2:16 OHE ratio and BMI.](image2)
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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