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Urinary estrogen metabolites and breast cancer risk in Chinese population

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Abstract

Background: In China, the association between estrogen metabolism and breast cancer risk and the differences in metabolic pattern between breast cancer patients and controls are poorly understood.

Methods: A total of 84 patients with invasive breast cancer and 47 controls with benign breast diseases were included in this study. Estrogen metabolites from their morning urine were determined by HPLC-MS/MS and evaluated in both groups, and the predictive value of each estrogen metabolite in the malignant group according to their menstrual status was analyzed.

Results: Urinary concentration of estrogen metabolites 2-hydroxyestrone (2-OHE1), 2-hydroxyestradiol (2-OHE2), 4-hydroxyestradiol (4-OHE2), 4-methoxyestrone (4-MeOE1), and 16 α -hydroxyestrone were lower in postmenopausal patients with breast cancer, compared with benign controls. In logistic regression model, breast cancer risk increased with the decline in the levels of 4-OHE2 and 4-MeOE1. In premenopausal patients, a difference in the level of 2-OHE2 was observed between both groups, and 2-OHE2 was found to have predictive value for breast cancer. Additionally, urinary 2-OHE2 level in premenopausal hormone receptor positive (HR+) patients was considerably higher compared with hormone receptor negative patients.

Conclusions: We found that lower urinary levels of 4-OHE2 and 4-MeOE1 had predictive value for breast cancer, and higher 2-OHE1 were associated with HR+ breast cancer in premenopausal women.

Key Words

- ▶ breast cancer
- ▶ liquid chromatography tandem mass spectrometry
- ▶ estrogen metabolism
- ▶ hormone receptor
- ▶ estrogen receptor

Endocrine Connections
(2021) 10, 1615–1622

Introduction

Breast cancer is a malignant tumor that occurs in the mammary gland. It is the leading female malignant tumor, posing a major threat to women's health globally. However, the incidence of breast cancer worldwide, including China, has gradually increased recently (1, 2).

In patients with breast cancer, abnormalities occur in estrogen metabolism, in particular in the level of estrogen and their metabolites. Hydroxylation of the carbon atoms at positions 2, 4, and 16 of the steroid ring of estrogen produces a cascade of metabolites with diverse

biological properties, such as 2-hydroxyestrone (2-OHE1), 4-hydroxyestrone (4-OHE1), and 16 α -hydroxyestrone (16 α -OHE1). These hydroxylated metabolites can be further methylated into less toxic methoxy derivatives or oxidized into mutagenic quinone adducts (3, 4, 5). Estrogen and their metabolites are considered to be one of the most important factors affecting breast cancer risk (6, 7). By binding to its corresponding estrogen receptor (ER), estrogen can activate the receptor signaling pathways to promote cell growth, proliferation, and endometrial

hyperplasia, ultimately leading to the occurrence of cancer (8, 9, 10). Estrogen metabolites cause noticeably different effects: 2-OHE1 and 2-hydroxyestradiol (2-OHE2), as tumor suppressors, can inhibit breast cancer cell growth and proliferation by reducing the binding affinity to the ER, in contrast to 16 α -OHE1 which has a very strong affinity to the ER with potential genotoxic damage (11, 12, 13, 14, 15).

Previous epidemiological studies of estrogen metabolism have been limited and were conducted using RIA and ELISA which have poor specificity, accuracy, and/or reproducibility (16, 17, 18, 19). HPLC coupled with MS makes more sensitive, specific, and accurate epidemiological studies possible (20, 21, 22, 23, 24, 25, 26).

Recently, we developed a rapid quantitative HPLC-MS/MS method that can simultaneously detect estrogen and their metabolites with high specificity (27). In this study, we used this method to determine estrogens and estrogen metabolites in the urine of patients with breast cancer and fibroadenoma, a benign breast disease. The association between ER status and estrogen metabolism in breast cancer was also analyzed.

Methods

Patients and samples

From January 2019 to September 2019, we collected morning urine samples from 84 invasive breast cancer patients and 47 breast fibroadenoma patients, in the Department of Breast Surgery, West China Hospital, Sichuan University. The inclusion criteria were as follows: (1) female patients aged 18–75 years old; (2) diagnosed with primary invasive breast cancer by core needle biopsy or diagnosed with fibroadenoma clinically; (3) have not received medical treatment of exogenous estrogen or progestin; (4) have not received systemic breast cancer-related treatment; and (5) have normal liver and kidney function. We excluded patients receiving exogenous estrogen or progestin or systemic treatment, exhibiting abnormal liver or kidney function, or diagnosed with another cancer, with endocrine disease, with ovary disease or pregnancy.

The morning urine samples were collected at the time of diagnosis for postmenopausal patients or on the 7th day in the menstrual cycle for premenopausal patients. ER positivity or progesterone receptor positivity was defined as both $\geq 1\%$ positive tumor cells with nuclear staining.

The study was approved by the Ethics Commission of West China Hospital of Sichuan University, and every participant signed an informed consent.

Laboratory assay

In urine, endogenous estrogens (estradiol and estrone) and their metabolites are present primarily in a conjugated form. Based on the criteria previously described, prior to sample analysis, a hydrolysis step is required to release endogenous estrogens and their metabolites in urine. Glucuronide and sulfate moieties were removed, allowing for the measurement of total levels of each estrogen and estrogen metabolite.

HPLC-MS/MS analysis was performed using a LCMS-8050 Triple Quadrupole Liquid Chromatography Mass Spectrometry system (Shimadzu, Kyoto, Japan) to measure estrogens and metabolites in urine samples (27). Quantification of estrogens and metabolites was carried out using LabSolutions software (Shimadzu). Molar quantities were standardized to creatinine accordingly.

Statistical analysis

Data were analyzed using SPSS version 25.0 (IBM). The chi-squared test was used to assess the association between breast diseases and the clinicopathological parameters of patients. The Mann–Whitney *U* test was applied for the comparison of estrogen metabolites between breast cancer and benign controls, and between patients who were hormone receptor (HR) positive or negative. Logistic regression was used to assess the association of estrogen metabolites that displayed significant differences between pairwise groups with breast cancer risk. The predictive value of estrogen metabolite for breast cancer was evaluated by receiver operating characteristic (ROC) curve.

Results

Baseline characteristics of patients

We analyzed 131 patients, including 84 diagnosed with invasive breast cancer and 47 diagnosed with breast fibroadenoma. The baseline characteristics of all patients are presented in Table 1, showing the association between the two groups in clinicopathological parameters. There were no significant differences between both groups in menstrual status and history of past illnesses (family history of breast cancer, history of smoking, or history

Table 1 Baseline characteristics.

Characteristic	Cases		Controls		P
	Number 84	%	Number 47	%	
Age (years)					0.005
<55	52	56.5	40	43.5	
≥55	32	82.1	7	17.9	
BMI (kg/m ²)					0.003
<25	48	55.2	39	44.8	
≥25	36	81.8	8	18.2	
Menopause					0.611
No	39	61.9	24	38.1	
Yes	45	66.2	23	33.8	
HR status					
Positive	66				
Negative	18				
Molecular subtype					
Luminal A BC	17				
Luminal B BC	49				
HER2-enriched	10				
TNBC	8				
Family history of breast cancer					1.000
No	78	63.9	44	36.1	
Yes	6	66.7	3	33.3	
History of smoking					1.000
No	76	63.9	43	36.1	
Yes	8	66.7	4	33.3	
History of alcohol consumption					1.000
No	77	64.2	43	35.8	
Yes	7	63.6	4	36.4	

Cases, invasive breast cancer; Controls, breast fibroadenoma; HR, hormone receptor; Luminal A BC, hormone receptor positive (ER and/or PR positive), HER2 negative, and Ki-67 low; Luminal B BC, hormone receptor positive (ER and/or PR positive), and either HER2 positive or HER2 negative with high levels of Ki-67; HER2-enriched, hormone receptor negative (ER and PR negative) and HER2 positive; TNBC, triple-negative breast cancer.

Bold indicates statistical significance, $P < 0.05$.

of alcohol consumption). We found that patients with increased age ($P=0.005$) or BMI ($P = 0.003$) were more likely to have breast cancer.

Estrogen metabolism in breast cancer and fibroadenoma patients

Due to the decrease in the levels of estrogen metabolites following menopause, cases were divided into premenopausal and postmenopausal groups for further analysis. The urinary concentrations of estradiol and estrone (parent estrogens) and their estrogen metabolites were compared in pre- or postmenopausal patients with breast cancer and breast fibroadenoma, respectively, and are presented in [Tables 2](#) and [3](#). In postmenopausal patients ([Table 3](#)), the urinary levels of 2-OHE1 ($P=0.029$), 2-OHE2 ($P=0.006$), 4-OHE2 ($P < 0.001$), 4-methoxyestrone (4-MeOE1) ($P < 0.001$), and 16 α -OHE1 ($P < 0.001$) in patients with breast cancer were remarkably lower compared with breast fibroadenoma counterparts. In premenopausal patients ([Table 2](#)), there was a significant difference in the urinary level of 2-OHE2 ($P=0.022$) between the two groups.

From the logistic regression analysis ([Fig. 1](#)), we found that 4-OHE2 and 4-MeOE1 were independent predictors for breast cancer risk. Patients with ≤ 0.085 ng/mg urinary 4-OHE2 and ≤ 0.115 ng/mg urinary 4-MeOE1 had a 9.9 and 8.5% reduced risk of developing breast cancer, respectively, compared with their counterparts. ROC analysis revealed that the levels of urinary 2-OHE1, 2-OHE2, 4-OHE2, 4-MeOE1, and 16 α -OHE1 in postmenopausal patients ([Fig. 2](#)) had predictive value for breast cancer, with the optimal cutoffs of 0.425, 0.415, 0.085, 0.115, and 0.345, respectively.

Table 2 Estrogen metabolism in premenopausal patients.

Estrogens and estrogen metabolites	Invasive breast cancer 50th (25th, 75th)	Breast fibroadenoma 50th (25th, 75th)	P
Parent estrogens	6.35 (4.29, 8.78)	4.45 (3.09, 8.59)	0.420
Estrone	4.09 (3.03, 5.29)	4.32 (2.79, 8.01)	0.466
Estradiol	2.04 (1.44, 3.84)	2.81 (1.54, 4.54)	0.305
2-Hydroxylation pathway	1.90 (1.21, 3.57)	3.11 (1.86, 6.19)	0.065
2-OHE1	1.03 (0.51, 2.49)	1.49 (0.56, 2.62)	0.484
2-OHE2	0.11 (0, 0.47)	0.73 (0, 1.44)	0.022
2-MeOE1	0.51 (0.36, 0.67)	0.58 (0.34, 0.92)	0.266
4-Hydroxylation pathway	0.31 (0.04, 0.82)	0.62 (0.11, 1.58)	0.152
4-OHE1	0.25 (0, 0.69)	0.34 (0, 1.43)	0.458
4-OHE2	0 (0, 0.06)	0 (0, 0.18)	0.113
4-MeOE1	0 (0, 0.01)	0 (0, 0.01)	0.708
16-Hydroxylation pathway	3.43 (1.93, 5.64)	4.11 (2.15, 10.42)	0.298
16 α -OHE1	0.54 (0.34, 0.98)	0.68 (0.31, 2.22)	0.226
Estriol	2.71 (1.55, 4.71)	3.11 (1.63, 8.02)	0.396
Total estrogens/estrogen metabolites	9.25 (7.87, 19.80)	10.17 (8.90, 23.45)	0.108

Bold indicates statistical significance, $P < 0.05$.

Table 3 Estrogen metabolism in postmenopausal patients.

Estrogens and estrogen metabolites	Invasive breast cancer 50th (25th, 75th)	Breast fibroadenoma 50th (25th, 75th)	P
Parent estrogens	3.14 (1.39, 4.86)	4.45 (2.93, 9.20)	0.464
Estrone	1.81 (0.76, 3.87)	3.11 (1.71, 5.25)	0.488
Estradiol	0.63 (0.31, 1.15)	1.54 (0.92, 3.45)	0.300
2-Hydroxylation pathway	1.09 (0.48, 2.21)	2.30 (1.53, 4.59)	0.028
2-OHE1	0.24 (0.02, 0.70)	0.83 (0.46, 1.87)	0.029
2-OHE2	0.32 (0.00, 0.92)	0.89 (0.34, 1.31)	0.006
2-MeOE1	0.25 (0.14, 0.51)	0.41 (0.26, 0.71)	0.071
4-Hydroxylation pathway	0.53 (0.09, 1.32)	0.87 (0.51, 1.50)	0.027
4-OHE1	0.45 (0.00, 1.32)	0.44 (0.24, 0.81)	0.539
4-OHE2	0.00 (0.00, 0.08)	0.13 (0.00, 0.24)	<0.001
4-MeOE1	0.00 (0.00, 0.11)	0.07 (0.00, 0.25)	<0.001
16-Hydroxylation pathway	1.08 (0.36, 1.84)	2.45 (0.78, 6.41)	0.297
16 α -OHE1	0.16 (0.06, 0.33)	0.58 (0.23, 1.89)	<0.001
Estriol	0.85 (0.26, 1.45)	1.89 (0.45, 5.18)	0.979
Total estrogens/estrogen metabolites	6.68 (4.15, 10.37)	12.15 (6.86, 24.53)	0.193

Bold indicates statistical significance, $P < 0.05$.

Association between hormone receptor status and estrogen metabolism

We next sought to determine the relationship between HR status and the levels of estrogen and their metabolites in premenopausal and postmenopausal patients (Table 4). In premenopausal patients with breast cancer, HR status was positively related to 2-OHE2 level ($P = 0.028$).

In contrast, there were no significant differences between HR status and all estrogen metabolites in postmenopausal patients (Supplementary Table 1, see section on [supplementary materials](#) given at the end of this article). ROC curve (Fig. 3) showed urinary 2-OHE2 had predictive value for HR status in premenopausal patients, with the optimal cutoffs of 0.21.

Figure 1

Forest plot of OR and 95% CI for estrogen metabolites. OR, odds ratios.

Figure 2

The ROC curve of urinary 2-OHE1 (area under curve = 0.662, $P = 0.03$), 2-OHE2 (area under curve = 0.703, $P = 0.006$), 4-OHE2 (area under curve = 0.847, $P < 0.001$), 4-MetOE1 (area under curve = 0.856, $P < 0.001$), and 16α-OHE1 (area under curve = 0.767, $P < 0.001$) in postmenopausal patients.

Discussion

Estrogen metabolism is the cumulative interaction of multiple pathways under the catalytic activities of numerous estrogen metabolic enzymes, during which a variety of metabolites are produced. Since these metabolites

exhibit pro- and antitumorigenic properties, an imbalance in the metabolites produced from these different metabolic pathways may lead to serious physiological changes and ultimately cancer. Therefore, understanding the estrogen metabolic pathways and exploring the mechanism of estrogen metabolism in tumorigenesis are of great

Table 4 Association between hormone receptor status and estrogen metabolism in premenopausal breast cancer patients.

Estrogens and estrogen metabolites	HR– 50th (25th, 75th)	HR+ 50th (25th, 75th)	P
Parent estrogens	7.10 (4.61, 11.35)	5.72 (3.71, 8.38)	0.382
Estrone	5.06 (3.02, 6.13)	3.85 (2.57, 4.85)	0.282
Estradiol	2.97 (1.59, 5.22)	1.94 (1.36, 3.30)	0.365
2-Hydroxylation pathway	2.64 (1.51, 3.56)	1.64 (1.21, 3.73)	0.708
2-OHE1	1.97 (0.38, 2.79)	0.87 (0.48, 2.18)	0.606
2-OHE2	0.00 (0.00, 0.08)	0.28 (0.00, 0.55)	0.028
2-MeOE1	0.55 (0.41, 0.77)	0.44 (0.29, 0.66)	0.288
4-Hydroxylation pathway	0.07 (0.00, 0.40)	0.44 (0.16, 0.94)	0.066
4-OHE1	0.07 (0.00, 0.40)	0.34 (0.00, 1.59)	0.145
4-OHE2	0.00 (0.00, 0.07)	0.32 (0.03, 0.88)	0.229
4-MeOE1	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.101
16-Hydroxylation pathway	3.43 (2.38, 5.64)	3.22 (1.87, 5.87)	0.864
16α-OHE1	0.67 (0.44, 1.20)	0.51 (0.34, 0.84)	0.473
Estriol	2.71 (1.64, 5.25)	2.61 (1.42, 5.32)	0.950
Total estrogens/estrogen metabolites	15.99 (10.23, 21.28)	12.11 (7.72, 22.08)	0.876

HR–, hormone receptor negative breast cancer; HR+, hormone receptor positive breast cancer.

Bold indicates statistical significance, $P < 0.05$.

Figure 3

The ROC curve of urinary 2-OHE2 (area under curve = 0.718, $P = 0.037$) for hormone receptor status in premenopausal patients.

importance to the effective prevention and treatment of breast cancer.

Estrogen metabolism involves a homeostatic state of active and inactive pathways. Aromatization of androstenedione and testosterone is catalyzed by cytochrome P450 (CYP) enzymes, yielding E1 and E2, respectively (28, 29, 30). Following the conversion from estradiol to estrone in the C17 position, hydroxylation occurs at the C2, C4, or C16 positions of estrone to form A-ring and D-ring metabolites (31), including 2-OHE1, 4-OHE1, 16 α -OHE1, and estriol. Previous studies have found that 4-OHE1 has a longer half-life *in vivo* than 2-OHE1 and is more easily oxidized into quinones, which physically bind to DNA, increasing the probability of DNA replication errors, and therefore increasing the risk for the occurrence of breast cancer.

In this study, we used a rapid HPLC-MS/MS approach to accurately detect and quantify urinary estrogen and estrogen metabolites in patients diagnosed with breast cancer and benign breast disease (due to limited urinary samples from healthy controls). The urine samples of premenopausal and postmenopausal patients were also analyzed. We observed significantly lower levels of urinary 2-OHE2 in premenopausal patients with invasive breast cancer than in patients with breast fibroadenoma. In postmenopausal patients, there was a statistical

difference in the levels of 2-OHE1, 2-OHE2, 4-OHE2, 4-MeOE1, and 16 α -OHE1 between both groups, showing these metabolites were considerably lower in the patients with breast cancer. The risk of developing breast cancer increased with a decline in the levels of 4-OHE2 and 4-MeOE1. Therefore, the correlation between the levels of estrogen metabolites and breast cancer risk was primarily analyzed in postmenopausal patients.

According to a comprehensive review, 4-OHE2 and 16 α -OHE1 are potential oncogenic molecular markers, 2-OHE1 and 2-OHE2 are primary estrogen metabolites, and estrogen methylation products, such as 2-MeOE1 and 4-MeOE1, are potential tumor suppressor molecular markers (15, 32, 33, 34, 35, 36, 37, 38). However, our results showed that the levels of 4-OHE2 (0.00 vs 0.13) and 16 α -OHE1 (0.16 vs 0.58) were lower in the patients with breast cancer and that increased 4-OHE2 may inhibit progression of breast cancer. There are a number of possible reasons for this discrepancy: first, the concentrations of estrogens are comparatively low in postmenopausal Asian women, particularly in China, which may influence the results; secondly, the sample size is limited, and large-scale prospective clinical studies are needed to validate our results; thirdly, our analysis included benign controls, rather than a healthy group, which may be inconsistent with the association in the metabolite levels between a malignant and normal group.

Few studies have focused on the association of estrogen metabolites in urine and tumor HR status. This study revealed that in premenopausal patients, 2-OHE2 in HR+ patients was considerably higher than in HR- patients, suggesting that the levels of 2-OHE2 could have different effects on HR+ and HR- breast cancers. Additionally, 2-OHE2 had predictive value for HE status for breast cancer patients, which was potentially attributed to the hypothesis that 2-OHE2 could promote HR+ breast cancer by binding with the HR to enhance nuclear mitosis of tumor cells. However, in postmenopausal patients, there was no difference between HR+ and HR- patients in estrogen and estrogen metabolites.

In summary, estrogen metabolism was associated with breast cancer risk in both premenopausal and postmenopausal women, and the level of 2-OHE2 could be an important factor for HR+ breast cancer.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EC-21-0226>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This study was funded by Science and Technology Department of Sichuan Province (No. 2019YF50377); 1.3.5 project for disciplines of excellence, West China Hospital, Sichuan University (No. ZYJC21082).

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Received in final form 19 October 2021

Accepted 22 November 2021

Accepted Manuscript published online 23 November 2021