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Risk of breast cancer associated with estrogen DNA adduct biomarker

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Abstract

Background: It is biologically plausible that genotoxic estrogens, namely estrogen DNA adducts (EDA), have a role in breast cancer development. Support comes from three prior studies which reported elevated concentrations of EDA relative to estrogen metabolites and conjugates (EDA:EMC) in women with breast cancer relative to control women.

Methods: In postmenopausal women in the Women's Health Initiative (WHI), EDA:EMC in 191 controls was compared to findings in 194 pre-diagnosis urine samples from breast cancer cases. EDA:EMC determinations were by mass spectrometry as previously described, and logistic regression was employed to estimate the odds ratios (OR).

Results: The EDA:EMC did not differ in breast cancer cases compared to controls overall (0.93 [95% CI: 0.71–1.23]), with a mean (SD) of 2.3 (0.8) and 2.4 (1.1) in cases and controls, respectively. Similarly, the ratio did not differ when examined by estrogen receptor or recency of biospecimen collection prior to breast cancer.

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Conclusion: Despite the demonstrated genotoxic properties of certain catechol estrogens resulting in estrogen DNA adducts, this analysis did not provide evidence for an increased breast cancer risk in relation to an elevated EDA:EMC.

Impact: This analysis conducted prospectively within post-menopausal women in the WHI study suggests that a strong association between EDA:EMC with breast cancer could be ruled out, as this study was powered to detect an OR of 2.2 or greater.

INTRODUCTION

Prior research suggests elevated concentrations of estrogen DNA adducts (EDA) may confer an increased breast cancer risk.[1] Three prior studies found that breast cancer cases had elevated concentrations of EDA relative to estrogen metabolites and conjugates (EDA:EMC), particularly for the 4-hydroxy pathway, compared to healthy controls in urine and blood samples.[2–,4] Moreover, these studies showed women at high versus low risk of breast cancer (defined by Gail score) had elevated EDA:EMC, suggesting that elevated EDA:EMC precedes cancer development.[2–,4] However, these studies had small sample sizes (<80 cases) and biospecimens in cases were not collected prior to breast cancer diagnosis. As these prior studies could not rule out that breast cancer itself led to elevated EDA:EMC, we conducted an analysis using prospectively-collected samples from the Women's Health Initiative (WHI) study in which EDA:EMC was examined in biospecimens collected prior to breast cancer.

MATERIALS and METHODS

The present analysis was conducted among 385 post-menopausal women from the WHI using a nested case-control design (n=191 cases; n=194 controls). Eligibility criteria included: aged 50–79 years at enrollment; enrollment in either the observational study or diet modification trial; and availability of biospecimen, which for cases must have been ≥1 year prior to diagnosis.[5] Cases had a physician-adjudicated diagnosis of incident breast cancer. Controls were 1:1 matched on age at enrollment, race/ethnicity, biospecimen collection timepoint, hysterectomy status, clinical site, and WHI study.[6] Subsequent to sample selection some participants were removed from analysis (i.e., removal of DCIS cases). *A priori* calculations indicated this study was powered to detect an OR=2.2. All participants provided informed consent. This study was approved by the Fred Hutch Cancer Research Center Institutional Review Board.

Laboratory methods were based on previous reports for EDA:EMC and creatinine,[4] with the exception of instrumentation and extraction methodology. Briefly, ascorbic acid was added to urine at collection, then urine was stored at −80°C. After thawing, a solution was made of 200*ul* of urine and 10*ul* of a 10ng/ml solution of 2-OH-3-OCH_3E_1 in water (as internal standard). The sample was vortexed for 15 seconds; 600*ul* of methanol was added; the sample was vortexed again for 30 seconds; the supernatant was removed and evaporated to dryness under a steady stream on nitrogen. The sample was then reconstituted with 50*ul* of a solution of 80:20 water:acetonitrile both containing 0.1% formic acid. 20*ul* injections were made using a Water's I-class UPLC platform coupled with a Water's Xevo TQ-S mass spectrometer.

Statistical analysis included unconditional logistic regression to estimate odds ratios (OR) of breast cancer associated with EDA:EMC. As previously described,[1–3] the numerator sums 6 EDA compounds (i.e., catechol estrogens in 4-hydroxy and 2-hydroxy pathways for both estrones and estradiols bound to a DNA base pair (Supplementary Table 1). The denominator sums estrogen metabolites and conjugates (i.e., those inactivated) in the same pathways but not bound to a DNA base pair (e.g., 2-OHE_2). The intra-assay coefficient of variation (CV) for all batches in this analysis was <30%. Secondary analyses included investigation of the 2- and 4-hydroxy pathways separately, and investigation of whether estrogen receptor (ER) status or a greater time between sample collection and cancer impacted results.

RESULTS

In this dataset, the mean age was 63 years, and women were predominantly white (Table 1). With the exception of Gail score, smoking history, and estrogen+progestin hormone therapy, cases and controls were similar. Overall, EDA:EMC did not differ between cases and controls (Table 2), and no difference in either EDA or EMC concentrations between cases and controls were seen. Risk did not differ when restricted to ER+ breast cancer, nor to those with a biospecimen collected ≥2 years prior to breast cancer.

DISCUSSION

In this analysis in which biospecimens were collected prior to cancer, we did not detect an association between EDA:EMC overall and breast cancer, nor an elevation in breast cancer risk associated with the 4-hydroxy or 2-hydroxy pathway when investigated separately.

The biologic plausibility for EDA's role in breast cancer has been described in numerous studies.[1,7,8] Certain catechol estrogen metabolites, particularly 4-hydroxy EDAs, are genotoxic as they are capable of forming DNA adducts, which through depurination may result in DNA mutation similarly to benzene, a known carcinogen.[1] Based on these pre-clinical observations, three human studies reported EDA:EMC was significantly elevated in women with breast cancer relative to control women.[1–3]

Limitations of this analysis include the relatively high %CVs for some compounds (Supplementary Table 1). The mean %CV across all compounds was 15%. For the most part, EDA compounds had lower %CVs and still those were not associated with elevated breast cancer risk. Prior to the present study, no study had evaluated this research question with prospectively-collected biospecimens, a strength of the present study. This study did not provide evidence that breast cancer risk was increased in relation to EDA:EMC, nor as hypothesized with an increased risk in relation to 4-hydroxy EDAs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CI	confidence interval
CV	coefficient of variation
DCIS	ductal carcinoma *in situ*
EDA	estrogen DNA adducts
EMC	estrogen metabolites and conjugates
ER	estrogen receptor
OR	odds ratio
SD	standard deviation
WHI	Women's Health Initiative

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Table 1.

Baseline Characteristics of breast cancer cases and controls

Characteristic	Case (n=191) n (%)[1]	Control (n=194) n (%)[1]
At Baseline		
Age, mean (SD)	63.0 (7.0)	62.9 (7.0)
Gail 5 year risk *		
<1.26	66 (34.6)	69 (35.6)
1.27 – 1.80	54 (28.3)	64 (33.0)
>1.80	71 (37.2)	61 (31.4)
Ethnicity		
White	154 (80.6)	157 (80.9)
Black	24 (12.6)	24 (12.4)
Hispanic	9 (4.7)	9 (4.6)
American Indian	3 (1.6)	3 (1.6)
Unknown	1 (0.5)	1 (0.5)
BMI, mean (SD)	28.4 (6.0)	28.6 (6.5)
Percent fat from whole body scan, mean (SD)	44.0 (6.7)	44.3 (7.7)
Smoking		
Never	92 (48.4)	100 (52.9)
Past	85 (44.7)	70 (37.0)
Current	13 (6.8)	19 (10.0)
Estrogen + progestin menopausal hormone therapy		
Never used	131 (68.6)	151 (77.8)
Past user	18 (9.4)	10 (5.2)
Current user	42 (22.0)	33 (17.0)
Unopposed estrogen menopausal hormone therapy		
Never used	127 (66.5)	126 (65.3)
Past user	27 (14.1)	27 (14.0)
Current user	37 (19.4)	40 (20.7)
Age at menopause, mean (SD)	48.7 (6.4)	47.8 (6.9)
Age at first birth		
Never pregnant /No term pregnancy	19 (10.8)	21 (12.4)
<20	29 (16.5)	27 (16.0)
20 – 29	116 (65.9)	110 (65.1)
30+	12 (6.8)	11 (6.5)
Parity (among those with term pregnancy)		
1–2	63 (36.6)	65 (37.6)
3–4	75 (43.6)	77 (44.5)
5+	34 (19.8)	31 (17.9)
Percent Calories from Fat, mean (SD)	33.7 (8.1)	33.7 (8.2)
WHI study		

Characteristic	Case (n=191) n (%)[1]	Control (n=194) n (%)[1]
Observational	119 (62.3)	121 (62.4)
Clinical Trial	72 (37.7)	73 (37.6)
Outcomes/Exposures		
Age at diagnosis,[2] mean (SD)	71.4 (7.8)	78.1 (7.4)
Estrogen receptor		
Positive	148 (77.5)	-
Negative	26 (13.6)	-
Unknown/Borderline	17 (8.9)	-
Progesterone receptor		
Positive	130 (68.1)	-
Negative	41 (21.5)	-
Unknown/Borderline	20 (10.5)	-
Her2 receptor		
Positive	21 (11.0)	-
Negative	107 (56.0)	-
Unknown/Borderline	14 (33.0)	-

* tertiled

1. Mean (SD) where indicated

2. For controls, age at diagnosis was calculated as age at last known follow-up date

Table 2.

Risk of breast cancer in relation to the EDA ratio[1]

	Case (n=191)		Control (n=194)		OR (95% CI)
	N	**%**	**N**	**%**	
Overall (N=385)					
EDA:EMC ratio					
Quartile 1	44	23.0	48	24.7	1.00 (Ref)
Quartile 2	54	28.3	49	25.3	1.57 (0.76,3.22)
Quartile 3	62	32.5	48	24.7	1.67 (0.82,3.41)
Quartile 4	31	16.2	49	25.3	0.95 (0.42,2.15)
	Mean	**SD**	**Mean**	**SD**	
EDA:EMC ratio, continuous[2]	2.3	0.8	2.4	1.1	0.93 (0.71,1.23)
EDA, continuous[2,3]	7.8	0.7	7.8	0.8	1.05 (0.74,1.49)
EMC, continuous[2,3]	13.4	0.6	13.3	0.6	1.26 (0.80,2.00)
4-catechol estrogen pathway EDA:EMC ratio	**N**	**%**	**N**	**%**	
Quartile 1	36	18.9	49	25.3	1.00 (Ref)
Quartile 2	58	30.4	48	24.7	1.95 (0.96,3.99)
Quartile 3	47	24.6	48	24.7	1.16 (0.55,2.47)
Quartile 4	50	26.2	49	25.3	1.68 (0.80,3.56)
	Mean	**SD**	**Mean**	**SD**	
4-EDA:EMC ratio, continuous[2]	1.2	1.0	1.2	1.3	1.04 (0.82,1.30)
2-catechol estrogen pathway EDA:EMC ratio	**N**	**%**	**N**	**%**	
Quartile 1	48	25.1	49	25.3	1.00 (Ref)
Quartile 2	63	33.0	48	24.7	1.92 (0.95,3.88)
Quartile 3	49	25.7	49	25.3	1.13 (0.55,2.34)
Quartile 4	31	16.2	48	24.7	0.81 (0.37,1.78)
	Mean	**SD**	**Mean**	**SD**	
2-EDA:EMC ratio, continuous[2]	1.6	0.9	1.8	1.0	0.84 (0.65,1.10)
Among ER+ breast cancer and matched controls (N=296)					
EDA:EMC ratio					
Quartile 1	34	23.0	37	25.0	1.00 (Ref)
Quartile 2	46	31.1	37	25.0	1.68 (0.74,3.80)
Quartile 3	46	31.1	37	25.0	1.56 (0.68,3.59)
Quartile 4	22	14.9	37	25.0	1.14 (0.44,2.95)
	Mean	**SD**	**Mean**	**SD**	
EDA:EMC ratio, continuous[2]	2.2	0.7	2.5	1.1	0.92 (0.66,1.28)
EDA, continuous[2,3]	7.8	0.6	7.8	0.9	1.07 (0.72,1.60)
EMC, continuous[2,3]	13.4	0.5	13.3	0.6	1.27 (0.74,2.16)

	Case (n=191)		Control (n=194)		OR (95% CI)
	N	%	N	%	
Among cases with biospecimen ≥2 years prior to diagnosis and matched controls (N=359)					
EDA ratio					
Quartile 1	41	23.0	46	25.4	1.00 (Ref)
Quartile 2	48	27.0	45	24.9	1.60 (0.75,3.41)
Quartile 3	57	32.0	44	24.3	1.84 (0.87,3.90)
Quartile 4	32	18.0	46	25.4	1.45 (0.62,3.37)
	Mean	**SD**	**Mean**	**SD**	
EDA:EMC ratio, continuous[2]	2.3	0.8	2.4	1.0	1.08 (0.80,1.47)
EDA, continuous[2,3]	7.8	0.7	7.8	0.8	1.22 (0.83,1.78)
EMC, continuous[2,3]	13.4	0.6	13.3	0.6	1.19 (0.73,1.93)

[1] Model adjusted for matching factors (randomization center, age, race/ethnicity, hysterectomy status, observational study enrollment), and covariates associated with the outcome: income, estrogen + progestin menopausal hormone therapy use, age at first period, age at menopause, percent body fat, total energy expenditure, dietary fiber, and Calcium + Vitamin D trial assignment

[2] Log-transformed

[3] Model additionally adjusted for creatinine.