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Urinary Biomarkers of Oxidative Stress and Breast Cancer Survival

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Abstract

PURPOSE—Systemic oxidative stress has been implicated in the pathogenesis and progression of many chronic diseases, including breast cancer. No studies have investigated F₂-isoprostanes (F₂-IsoPs), valid biomarkers of systemic oxidative stress, in association with breast cancer prognosis. We conducted a nested case-control study in a prospective breast cancer survivor cohort to investigate systemic oxidative stress and survival.

METHODS—Urinary levels of F₂-IsoPs and its major urinary metabolite (2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP, F₂-IsoP-M) were measured post-cancer treatment using gas chromatography/negative ion chemical ionization mass spectrometry for 57 deceased breast cancer patients (cases) and 103 surviving patients (controls) matched 1:2 on age at diagnosis, stage, and diagnosis year. Odds ratios (ORs) and 95% confidence intervals (CIs) were derived from conditional logistic regression models.

RESULTS—In unadjusted models, elevated F₂-IsoP levels categorized based on the median value (1.73;<1.73 (reference)) were non-significantly inversely associated with mortality (OR: 0.51, 95% CI:0.24–1.10). After adjustment for potential confounders, elevated F₂-IsoP levels were significantly associated with mortality (OR:0.36, 95% CI:0.14–0.96). The inverse association was marginally significant when F₂-IsoP was categorized based on tertiles ($P_{\text{trend}}=0.08$). In contrast, elevated F₂-IsoP-M levels, categorized based on the median level (0.91;<0.91(reference)), were associated with a statistically non-significant increased risk of mortality in both unadjusted and adjusted models (adjusted OR:1.39, 95% CI:0.62–3.09).

CONCLUSION—Results suggest a role for oxidative stress biomarkers in breast cancer survival; however, as this is the first study to date, additional larger studies are needed.

Keywords

oxidative stress; F₂-isoprostanes; breast cancer; survival; prognosis

INTRODUCTION

Systemic oxidative stress has been implicated in the pathogenesis and progression of many chronic diseases, including breast cancer. Many cancer treatments including radiotherapy and chemotherapy regimens involve the generation of free radicals (e.g., hydroxyl radical, hydrogen peroxide, and superoxide anion) to induce oxidative stress to kill cancer cells [1, 2]. While oxidative stress mechanisms play a key role in cancer treatment, high levels of free radicals may negatively impact breast cancer prognosis through several mechanisms, including genomic instability potentially leading to treatment resistance, activation of cell signaling pathways involved in tumor cell proliferation, and increased tumor cell migration and pro-angiogenic factors [1, 3, 4]. We hypothesized that high systemic levels of oxidative stress after completion of chemotherapy and radiotherapy may negatively impact breast cancer prognosis.

Data on systemic oxidative stress biomarkers and breast cancer prognosis is very limited [5, 6]. F₂-isoprostanes (F₂-IsoPs), and one of its major metabolites, 2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP (F₂-IsoP-M), are considered valid biomarkers of systemic *in vivo* oxidative stress, with higher levels indicating increased systemic oxidative stress [7–10]. These prostaglandin-like compounds are formed from the free-radical catalyzed peroxidation of arachidonic acid [7]. To our knowledge, no study has investigated the association between F₂-IsoPs or F₂-IsoP-M levels and breast cancer survival. We conducted a nested case-control study in the Shanghai Breast Cancer Survival Study (SBCSS) to investigate the association of oxidative stress biomarkers after primary cancer treatment with (1) clinical and lifestyle characteristics and (2) mortality among breast cancer patients.

METHODS

Study Design and Population

We conducted a nested case-control study in the SBCSS, a prospective cohort of breast cancer patients. Details of the SBCSS methodology have been described [11]. Briefly, female permanent residents of Shanghai aged 20–75 years newly diagnosed with primary breast cancer between March 2002 and April 2006 were identified from the Shanghai Cancer Registry and approached for participation approximately six months after diagnosis. Of 6,299 eligible cases, 5,042 participated (80.0%) and provided written informed consent. Baseline interviews collected information on cancer treatment, tumor characteristics, reproductive history, diet, medical history, selected lifestyle factors, and socio-demographics. Medical records were reviewed to verify clinical data. Outcome data were ascertained via follow-up interviews and linkage with the Shanghai Vital Statistics Registry. Human subjects Institutional Review Board approval was obtained from all participating institutions.

We first identified 1,424 SBCSS participants who had provided a urine sample during their participation in the Shanghai Breast Cancer Study (SBCS), a population-based case-control study with overlapping recruitment with the SBCSS between 2002–2005 (Supplemental Figure S1). Women with TNM stage 0 (n=38), stage IV (n=10), stage unknown (n=55), or with a urine sample collected during chemotherapy and/or radiotherapy (n=593) were excluded. Among the 728 eligible women, 57 died (before the last follow-up date (i.e., last in-person interview date or last registry linkage date); 87.2% due to breast cancer). Two controls (surviving breast cancer patients) per case were randomly selected, matched on age at diagnosis (+/- 1 year), TNM stage (I, II, III), and year of diagnosis. For 11 cases only 1 control was available, resulting in 57 cases and 103 controls for the present study.

Measurement of Oxidative Stress Biomarkers

At time of interview for the case-control study, women provided a spot urine sample. The samples were collected into a sterilized cup containing 125 mg ascorbic acid. After collection, samples were kept cold (~0–4 °C) and processed within 6 hours and stored at -80 °C. Fourteen quality control samples were included. Laboratory staff were blinded to both the quality control and case-control status of the samples. Urinary levels of F₂-IsoPs and F₂-IsoP-M were quantified by gas chromatography/negative ion chemical ionization mass spectrometry (GC/NICI MS) using validated assays established by the Vanderbilt Eicosanoid Core Laboratory [12, 13]. The limit of detection for the assay is approximately 5 pg [13]. Biomarkers were adjusted for creatinine concentrations and expressed as ng/mg of creatinine. The inter-assay coefficient of variation was <8% for both biomarkers.

Statistical Analysis

Participants with undetectable values were excluded from the analysis for the specific biomarker (F₂-IsoPs - (3 cases and 5 controls), F₂-IsoP-M (1 case)). Clinical characteristics and lifestyle factors investigated in association with the biomarkers included age at diagnosis, education, income, chemotherapy, radiotherapy, tamoxifen therapy, menopausal status, ER/PR status, exercise participation, BMI, vitamin supplement use (any type or vitamin C or E supplement use), ginseng use, tea consumption, smoking, soy isoflavones intake (mg/day in tertiles), and cruciferous vegetables, meat, and fish intake (g/day in tertiles). Few women in Shanghai drink alcohol regularly [14], and in the overall SBCSS cohort <0.3% reported drinking alcohol at the post-diagnosis interview, and none of the participants in the nested case-control study were current alcohol drinkers. Biomarkers were logarithmically transformed to mitigate skewness in distributions (for use in general linear models). Geometric means and 95% confidence intervals (CIs) for F₂-IsoPs and F₂-IsoP-M were derived from least square means estimated using general linear models.

Odds ratios (ORs) and 95% CIs were derived from conditional logistic regression models, conditioned on matching factors. Potential confounding factors/known prognostic factors included: chemotherapy, radiotherapy, tamoxifen, ER/PR status, weeks between diagnosis and urine collection, weeks between end of cancer treatment and urine collection, education, income, BMI, menopausal status, and vitamin supplement use. Adjustment for time of day of sample collection, hours since last meal, and days since last menstrual cycle did not alter findings, and these factors were not included in final models. We also re-ran the analysis

including women with undetectable biomarker values in the lowest category and results did not differ from those presented (data not shown). Analyses were performed using SAS version 9.3. Tests of statistical significance were based on two-sided probability and p-values <0.05 were considered statistically significant.

RESULTS

Characteristics of cases and controls are displayed in Table 1. Compared to controls, cases had a higher BMI and were less likely to use vitamin supplements after diagnosis. Mean (SD) levels of F₂-IsoPs were 1.92 (1.0) in cases and 2.07 (1.1) in controls. Mean (SD) levels of F₂-IsoP-M were 1.06 (0.56) in cases and 0.98 (0.47) in controls. The correlation coefficient between F₂-IsoPs and F₂-IsoP-M was 0.39. A similar correlation between these two biomarkers was observed in the Shanghai Women's Health Study (correlation coefficient= 0.34) [15].

In adjusted models, postmenopausal status and vitamin C supplement use were significantly associated with higher F₂-IsoPs levels (Table 2). In adjusted models, later TNM stage and use of vitamin E supplements after diagnosis were significantly associated with lower F₂-IsoP-M levels (Table 2). Time between end of cancer treatment and urine collection, smoking status, and additional dietary factors (i.e., soy isoflavones intake, cruciferous vegetable intake, meat intake, fish intake, tea consumption, and ginseng use) were not significantly associated with either biomarker (data not shown).

Table 3 displays associations for F₂-IsoPs and F₂-IsoP-M with survival. In unadjusted models, categorized based on the median level (1.73;<1.73(reference)), F₂-IsoPs levels were not significantly associated with survival. After adjustment for potential confounding factors, elevated F₂-IsoPs levels were significantly inversely associated with mortality (OR: 0.36, 95% CI: 0.14–0.96). The inverse association was marginally significant when F₂-IsoPs levels were categorized based on tertiles (P_{trend}=0.08). In contrast, elevated F₂-IsoP-M levels, categorized based on the median level (0.91;<0.91 (reference)), were associated with a statistically non-significant increased risk of mortality in unadjusted models (OR: 1.36, 95% CI: 0.68–2.71), and the association was similar after adjustment for potential confounding factors. As described above, time between end of treatment (chemotherapy and radiotherapy) and urine collection was not associated with the two biomarkers. Regardless, we conducted a sensitivity analysis adjusting for the time between chemotherapy end date and date of urine collection among women who received chemotherapy (92% of the study population), and results were similar to overall findings (data not shown). Few women reported smoking (4%) and findings were similar after adjustment for smoking status (data not shown).

DISCUSSION

To our knowledge, this is the first study to investigate systemic biomarkers of oxidative stress after cancer treatment in association with breast cancer survival. In this prospective nested case-control study among Chinese breast cancer patients, we observed a statistically significant inverse association between F₂-IsoPs and all-cause mortality after adjustment for

potential confounders, with a marginally significant trend ($P_{\text{trend}}=0.08$). In contrast, for F₂-IsoP-M, we observed a non-significant positive association with mortality.

Very few studies have reported on the association of biomarkers of oxidative stress and breast cancer outcomes, and no studies have investigated F₂-isoprostanes. We identified a study among breast cancer patients diagnosed 1989–1992 that reported increased risk of recurrence in association with elevated MDA formation, a marker of lipid peroxidation [5]. However, low specificity is a major concern for this biomarker. In 2010, Sova and colleagues evaluated the association between pre-operative serum 8-oxodG levels and tumor aggressiveness (e.g., lymphatic invasion, nodal status) and breast cancer-specific survival. They reported that low levels of 8-oxodG were associated with tumor aggressiveness, but not survival [6]. One issue with 8-oxodG as an oxidative stress biomarker is that it is affected by DNA repair capacity, and therefore low levels may not necessarily reflect low oxidative damage, but instead impaired DNA repair [6]. In 2011, Vera-Ramirez and colleagues reported no association between DNA single-strand breaks (assessed via the comet assay) or plasma levels of protein carbonyl groups (marker of protein oxidation) with either disease-free or overall survival among 70 non-metastatic breast cancer patients [16].

Our finding of an inverse association between post-treatment levels of F₂-IsoPs and overall mortality was in contrast to our hypothesis, which was that high levels of oxidative stress after completion of chemotherapy and radiotherapy would be associated with poorer prognosis. While some researchers have postulated lipid peroxidation may play a potential protective role in breast cancer etiology, the biological mechanisms are far from clear [17]. Further, while we might expect high levels of oxidative stress during radiotherapy or chemotherapy to be associated with improved outcomes, as a potential reflection of treatment response, our samples were collected after completion of treatment, as we were interested in the role of systemic oxidative stress in breast cancer metastasis and survival after primary cancer treatment. Further, high levels of oxidative stress during treatment are not necessarily beneficial and oxidative damage may result in (1) increased genomic instability, potentially leading to treatment resistance and/or (2) treatment toxicity, which could affect ability to tolerate treatment in the short-term and potentially have long-term effects on normal tissues [1, 18]. Time between end of treatment (chemotherapy and radiotherapy) and urine collection was not associated with the two biomarkers in our study. This result matches findings from a study that measured urinary levels of F₂-isoprostanes before, during, and after chemotherapy, and reported that oxidative stress levels returned to baseline levels in 24 hours [19]. Future studies that measure biomarkers both during and after specific cancer treatments are needed.

We identified only one study on the association of F₂-isoprostanes with clinical characteristics and modifiable factors among breast cancer survivors [20]. In this study of 179 women from the Women's Healthy Eating and Living Study, post-cancer treatment urinary levels of F₂-IsoPs (measured via immunoassay) and 8-oxodG were inversely associated with vitamin E dietary intake, while F₂-IsoPs levels were associated with increased BMI and polyunsaturated fatty acid intake. The mean value for F₂-IsoPs was 1.80 ng/mg creatinine, similar to our measurement. In our study, we did not observe a significant association for BMI and the two biomarkers in adjusted models. However, vitamin E

supplementation was inversely associated with F₂-IsoP-M, but not F₂-IsoPs. Similarly, a study in the Shanghai Women's Health Study reported that vitamin E supplementation, and also plasma levels of alpha-tocopherol, were significantly inversely associated with F₂-IsoP-M, but not F₂-IsoPs [15].

A major strength of our study was assessment of oxidative stress using urinary levels of F₂-IsoPs and F₂-IsoP-M, which are considered valid biomarkers of total systemic oxidative stress *in vivo* [7, 8]. Urine measurement is preferred as the F₂-IsoPs are not subject to artifactual generation by autoxidation of arachidonic acid during processing and storage [7]. We measured both F₂-IsoPs and a major metabolite, as the metabolite is not affected by local renal production of F₂-IsoPs [7, 9]. Furthermore, we quantified the compounds using GC/NICI MS, a method that has very high sensitivity and specificity [13].

It is important to note that this is a first preliminary investigation of these biomarkers of oxidative stress and survival among breast cancer patients. It is possible that our results could be due to chance, and additional studies with a larger sample size are needed to confirm findings. Another limitation of our study is the use of only one spot urine sample. While measurement of F₂-IsoPs and F₂-IsoP-M based on spot urine samples has been shown to have low intra-individual variability over one year [21], in cancer patients it may be of interest to measure these biomarkers during cancer treatments and after treatment, to provide a more comprehensive assessment of the role of oxidative stress in cancer prognosis. Although we considered many potential confounding factors including clinical factors, socio-demographics, and lifestyle factors, we cannot exclude the possibility of residual confounding from inadequately measured covariates or unmeasured confounders.

In summary, in the first investigation to date, we observed that elevated F₂-IsoPs levels were significantly inversely associated with mortality, while elevated F₂-IsoP-M levels were non-significantly positively associated with mortality. Our results suggest a role for urinary oxidative stress biomarkers in breast cancer survival. However, results from this preliminary first study require confirmation in future larger studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

F₂-IsoPs	F ₂ -Isoprostanes
F₂-IsoP-M	2,3-dinor-5,6-dihydro-15-F _{2t} -IsoP
8-oxodG	8-hydrodeoxyguanosine
BMI	body mass index
CI	confidence interval
CV	coefficient of variation
ER	estrogen receptor
MDA	malondialdehyde
OR	odds ratio
PR	progesterone receptor
SBCS	Shanghai Breast Cancer Study
SBCSS	Shanghai Breast Cancer Survival Study

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Table 1

Distribution of matching factors, socio-demographics, clinical characteristics, and post-diagnosis lifestyle factors at baseline 6-month post-diagnosis interview by case/control status, N=160

	Controls (n = 103)		Cases (n = 57)	
Age at Diagnosis , median (25–75 th percentile)	51.7	(45.8–62.4)	51.6	(45.7–61.8)
Stage , n (%)				
I	16	(15.5)	8	(14.0)
II	69	(67.0)	36	(63.2)
III	18	(17.5)	13	(22.8)
Education , n (%)				
<High school	44	(42.7)	28	(49.1)
High school	46	(44.7)	22	(38.6)
>High school	13	(12.6)	7	(12.3)
Income (yuan/month) , n (%)				
<1000	61	(59.2)	39	(68.4)
1000	42	(40.8)	18	(31.6)
Chemotherapy , n (%)	96	(93.2)	52	(91.2)
Radiotherapy , n (%)	29	(28.2)	10	(17.5)
Tamoxifen , n (%)	58	(56.3)	35	(61.4)
Post-menopausal , n (%)	52	(50.5)	28	(49.1)
ER/PR status , n (%)				
ER+/PR+	53	(51.5)	27	(47.4)
ER–/PR–	31	(30.1)	19	(33.3)
ER–/PR+ or ER+/PR–	19	(18.5)	11	(19.3)
Regular exercise participation , n (%)	69	(67.0)	36	(63.2)
BMI (kg/m²) , n (%)				
<25	64	(62.1)	36	(63.2)
25–30	36	(35.0)	14	(24.6)
30	3	(2.9)	7	(12.3) ^a
Regular vitamin supplement use , n (%)	39	(37.9)	11	(19.3) ^a
Regular antioxidant supplement use , ^b n (%)				
Never	64	(62.1)	46	(80.7)
Yes	31	(30.1)	7	(12.3)
Other	8	(7.8)	4	(7.0) ^a
Regular ginseng use , n (%)	14	(13.6)	8	(14.0)
Weeks between diagnosis and urine collection , Median (25–75 th percentile)	18.9	(17.6–26.0)	18.7	(17.7–22.6)
Weeks between end treatment date and urine collection , ^c Median (25–75 th percentile)	4.9	(1.9–7.6)	4.2	(1.9–6.4)

^a P-value <0.05.

^b Antioxidants included intakes of vitamin C, vitamin E, and multivitamins.

^c Time between end treatment date and urine collection is missing for those who did not have radiotherapy/chemotherapy (n=13).

Table 2

Adjusted geometric means (95% CIs) for urinary excretion of F₂-IsoPs and F₂-IsoP-M by socio-demographics, clinical characteristics, and select lifestyle factors, N=160

	N	F ₂ -IsoPs ^a	F ₂ -IsoP-M ^b
Age at diagnosis			
<40	7	1.71 (1.10–2.65)	0.64 (0.44–0.91)
40–<50	65	1.83 (1.55–2.17)	0.92 (0.80–1.06)
50–<60	38	1.96 (1.65–2.34)	0.81 (0.70–0.94)
60	49	1.53 (1.24–1.89)	1.01 (0.85–1.20)
Education			
<High school	71	1.97 (1.73–2.24)	0.95 (0.85–1.07)
High school	68	1.60 (1.39–1.83)	0.88 (0.78–0.98)
>High school	20	1.65 (1.31–2.08) ^d	0.82 (0.67–1.00)
Income			
<1000	99	1.77 (1.59–1.97)	0.91 (0.83–1.00)
1000	60	1.75 (1.52–2.01)	0.89 (0.79–1.00)
Chemotherapy			
No	12	1.83 (1.36–2.46)	1.02 (0.78–1.32)
Yes	147	1.76 (1.61–1.91)	0.89 (0.83–0.96)
Radiotherapy			
No	120	1.74 (1.58–1.91)	0.94 (0.86–1.02)
Yes	39	1.83 (1.55–2.17)	0.81 (0.70–0.93) ^d
Tamoxifen			
No	66	1.68 (1.47–1.92)	0.89 (0.80–0.99)
Yes	93	1.82 (1.63–2.04)	0.91 (0.83–1.00)
TNM stage			
I	24	1.87 (1.51–2.31)	0.98 (0.81–1.18)
II	104	1.69 (1.53–1.88)	0.94 (0.87–1.03)
III	31	1.92 (1.59–32)	0.73 (0.63–0.86) ^c
Menopausal status			
Premenopausal	80	1.51 (1.29–1.76)	0.88 (0.77–1.00)

	N	F ₂ -IsoPs ^a	F ₂ -IsoP-M ^b
Postmenopausal	79	2.07 (1.77–2.42) ^c	0.93 (0.81–1.06)
ER/PR status			
ER+/PR+	80	1.68 (1.50–1.89)	0.88 (0.80–0.98)
ER-/PR-	49	1.98 (1.71–2.29)	0.92 (0.81–1.05)
ER-/PR+ or ER+/PR-	30	1.64 (1.36–1.99) ^d	0.93 (0.79–1.09)
Exercise			
No	55	1.69 (1.48–1.95)	0.89 (0.79–1.00)
Yes	104	1.80 (1.63–1.99)	0.91 (0.83–0.99)
BMI			
<25	100	1.78 (1.60–1.97)	0.87 (0.80–0.95)
25–30	49	1.81 (1.55–2.10)	0.96 (0.84–1.09)
30	10	1.46 (1.05–2.01)	0.97 (0.73–1.29)
Vitamin supplement use			
No	110	1.69 (1.53–1.86)	0.92 (0.84–1.00)
Yes	49	1.93 (1.67–2.24)	0.88 (0.77–1.00)
Vitamin C use			
No	110	1.69 (1.53–1.86)	0.92 (0.84–1.00)
Yes	35	2.08 (1.75–2.48) ^c	0.91 (0.78–1.06)
Vitamin E use			
No	110	1.69 (1.53–1.87)	0.92 (0.84–1.00)
Yes	25	1.72 (1.39–2.13)	0.75 (0.63–0.90) ^c

^a Adjusted for age, menopausal status, education, ER/PR status, BMI, vitamin supplement use, and assay batch. Additional 7 women missing F₂-IsoPs were excluded.

^b Adjusted for age, menopausal status, education, BMI, vitamin supplement use, and assay batch.

^c P-value <0.05.

^d P-value <0.10.

ORs and 95% CIs for Risk of Total Mortality in Association with Urinary Excretion of F₂-IsoPs and F₂-IsoP-M (ng/mg of creatinine), N=160

Table 3

	Cases/Controls	Model 1 ^a		Model 2 ^b		Model 3 ^c	
		OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
F₂-IsoPs^d							
<1.725	32/43	1.00	(reference)	1.00	(reference)	1.00	(reference)
1.725	22/51	0.51	(0.24–1.10)	0.44	(0.19–1.03)	0.36	(0.14–0.96)
F₂-IsoPs^d							
<1.48	20/29	1.00	(reference)	1.00	(reference)	1.00	(reference)
1.48–<2.07	19/28	0.78	(0.31–1.97)	0.75	(0.28–2.00)	0.58	(0.20–1.67)
2.07	15/37	0.47	(0.17–1.32)	0.40	(0.13–1.20)	0.35	(0.11–1.13)
P _{trend}			0.13		0.08		0.08
F₂-IsoP-M^e							
<0.908	26/53	1.00	(reference)	1.00	(reference)	1.00	(reference)
0.908	30/49	1.36	(0.68–2.71)	1.30	(0.63–2.68)	1.39	(0.62–3.09)
F₂-IsoP-M^e							
<0.745	17/35	1.00	(reference)	1.00	(reference)	1.00	(reference)
0.745–<1.07	16/35	1.12	(0.48–2.60)	1.13	(0.46–2.79)	0.97	(0.38–2.50)
1.07	23/32	1.76	(0.73–4.23)	1.58	(0.62–4.02)	1.89	(0.67–5.32)
P _{trend}			0.20		0.33		0.22

^aModel 1: ORs and 95% CIs are from conditional logistic regression models (matching factors include age, stage, and year of diagnosis).

^bModel 2: Also adjusted for clinical factors (chemotherapy, radiotherapy, tamoxifen, ER/PR status), weeks between diagnosis and urine collection.

^cModel 3: Also adjusted for education, income, BMI, menopausal status, and vitamin supplement use.

^dExcludes five controls and three cases missing F₂-IsoPs (and matched controls to the three cases (n=4)).

^eExcludes 1 case missing F₂-IsoP-M (and the matched control (n=1)).