Article

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Association between Urinary Lead and Female Breast Cancer: A Population-Based Cross-Sectional Study

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Background: Previous studies have explored the relationship between serum lead levels and the risk of female breast cancer (FBC). However, it is still uncertain whether urinary lead levels are associated with FBC. This study aimed to investigate the potential association between urinary lead and FBC.

Methods: A cross-sectional case-control study was conducted using the National Health and Nutrition Examination Survey (NHANES), which is a series of cross-sectional, nationally representative surveys of the United States population consisting of 10 survey waves from 1999 to 2018. This study analyzed a total of 2795 female participants (≥20 years), consisting of 210 participants with FBC and 2585 healthy controls. Urinary lead was detected using Inductively Coupled Plasma-Mass Spectrometry, which was divided into four levels by using quartiles-defining cut points. Multivariate logistic regression was used to analyze the association between urinary lead and FBC.

Results: Multivariate logistic regression revealed that urinary lead was positively correlated with FBC (Odds ratio [OR], 2.16; 95% confidence interval [CI]: [1.18, 3.95], $p < 0.05$) in a fully adjusted model. There were significantly increased ORs of FBC in quartile 4 (Q4) and quartile 3 (Q3), compared with the lowest quartile 1 (Q1) (Q4, OR = 1.48, 95% CI [0.89, 2.48]; Q3: OR = 1.01, 95% CI [0.59, 1.73], p for trend = 0.021). No significant interaction effects were observed between urinary lead levels and FBC between the subgroups (age, race, educational status, body mass index (BMI), marital status, family income to poverty ratio, hypertension status, diabetes status, renal function status, smoking history, ever been pregnant, oral contraceptive use, occupation classification, etc.) (All interaction p-value > 0.05).

Conclusions: Urinary lead is likely positively associated with FBC in the US population.

Keywords: female breast cancer (FBC); lead exposure; urinary biomarkers; cross-sectional study; NHANES

Introduction

Lead is a toxic environmental metal that comes from a variety of sources, including paint, industrial emissions, e-waste, herbal products, traditional medicines, water, soil, and air [1]. While Western countries have achieved considerable improvement in environmental lead contamination through strict control strategies, it remains a problem in city centers and several developing countries [2]. Humans are mainly exposed to lead through ingestion of contaminated food, water, and utensils, as well as inhalation of lead-contaminated polluted air dust and aerosols [3,4]. Evidence shows that 99% of lead intake is via ingestion, while inhalation contributes only 1% of lead intake [5]. Ingestion of lead-contaminated food is a considerable threat to humans, and currently, control over lead contamination from diverse sources in global food is still not ideal [6]. Therefore, understanding lead exposure in the human body is imperative.

Lead exposure can cause pathological alterations in most tissues and organs throughout the body in humans [7,8], which can influence neurological development in children and cause damage to the reproductive system, and hematologic system, while also inducing cardiovascular diseases, diabetes, and tumorigenesis [4]. Lead has the features of metalloestrogens, which may have possible endocrine-disrupting effects or suspected carcinogens [9]. It could mimic the "estrogen-like effects" and activate estrogen receptor α, inducing the proliferation of estrogen-dependent breast cancer cells, and increasing the expression of estrogen-regulated genes [9,10]. Lead may promote cancer formation, especially which can significantly increase the risk of progressing to hormone-dependent breast cancer [11]. A case-control report indicated the expression of

lead in breast cancer tissues was higher than that in normal breast tissues, which increased the risk of breast cancer [12]. Heavy metals (including lead) exposure can disturb human metabolomics, contributing to morbidity and even mortality [6].

Epidemiological data suggest that lead concentrations in the human body depend on age, place of residence, and lifestyle [13]. Most humans are exposed to low-to-moderate levels of environmental lead, as previous studies have shown that blood lead concentrations signify recent exposure, while increased urinary lead concentration means long-term chronic exposure. When the blood lead concentration reaches critical levels or renal-tubular damage, it can lead to long-term chronic exposure. For that reason, exploring the relationship between urinary lead and breast cancer has become important [14,15]. However, studies on the association between urinary lead and breast cancer have been sparse and inconsistent in the past years [16,17]. In this current study, a cross-sectional case-control design was implemented to discuss the independent association between urinary lead and female breast cancer (FBC).

Materials and Methods

Study Design and Participants

This cross-sectional case-control study included data on female participants ($\geq$20 years of age) during the 10 cycles of National Health and Nutrition Examination Survey (NHANES) from 1999–2018. NHANES is a nationally representative, cross-sectional study performed since 1999 by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention. This study aims to assess information on the health and nutritional status of the noninstitutionalized civilian population in the United States.

Urinary Lead Measurement

All urine specimens collected from participants were processed, stored, and shipped to the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention for analysis. The lead concentrations in urine specimens were measured using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The laboratory results meet the Division of Laboratory Science's quality control and quality assurance performance criteria for accuracy and precision, which are similar to the Westgard rules.

Diagnosis and Assessment of Breast Cancer

In this study, self-reported diagnoses of cancer status were obtained using medical conditions questionnaires. All participants were asked if they had ever been told by a doctor or other health professional that they had cancer or a malignancy of any kind. Those who replied "No cancer" were categorized as control participants, while those who replied "Yes" were asked what kind of cancer it was. Those who replied "breast cancer" were coded as the breast cancer group.

Covariates

We considered age, body mass index (BMI, kg/m^2), race/ethnicity, marital status, educational status, poverty status, hypertension status, renal function status, physical activity, reproductive health conditions (including age at menarche, being pregnant, oral contraceptive use, ever use female hormones), smoking history and occupation classification as major potential confounding factors. Race/ethnicity was divided into the following categories: Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, Other Race - Including Multi-Racial. Marital status was categorized as either sexual partner or asexual partner. The ratio of family income to poverty was categorized as being less than 1 and equal to or greater than 1. Hypertension status was categorized as having or not having hypertension. The renal function was categorized as either weak or failing kidneys, or healthy kidneys. Diabetes status was divided into three categories: no diabetes, having diabetes, and prediabetes. Physical activity was classified as either moderate/vigorous recreational activities or none. Smoking history was categorized as having smoked at least 100 cigarettes in life and never having smoked. The occupation of included participants was classified into private wage, government-employed, self-employed, and others. Lastly, the model also took into account reproductive health conditions, including age at menarche ($<$12 years or $\geq$12 years), ever been pregnant (no or yes), oral contraceptive use (no or yes), and female hormones (no or yes).

Statistical Analyses

Continuous variables were expressed as means $\pm$ standard deviation (SD) or medians with ranges, and categorical variables were expressed as percentages. The Kolmogorov–Smirnov test was used to verify whether the data conformed to a normal distribution. The *t*-test (for normal distribution) or Welch Two Sample *t*-test (for skewed distribution) was used for continuous variables, and the χ^2 test (or Fisher's exact test) for categorical variables to evaluate the baseline differences. Moreover, the univariable regression model was used to assess the association between baseline characteristics and FBC, and the multivariable regression model was further conducted to assess the independent effect of urinary lead on FBC. A stratified analysis in different subgroups was conducted using logistic regression models. Interaction tests were used to determine the difference among different stratifications in the subgroups, with results displayed as Odds ratios (ORs) in a Forest plot. ORs (95% confidence interval (CI)) were used to assess the association between urinary lead and FBC in the unadjusted and adjusted models using logistic regression. All statistical analyses were performed using Empower software version

2.0 (https://www.empowerstats.com; X&Y Solutions, Inc., Boston, MA, USA) and R version 3.4.3 (http://www.R-project.org, R Foundation for Statistical Computing, Vienna, Austria). *p* values less than 0.05 were considered statistically significant.

Patient and Public Involvement

We utilized publicly available data from the NHANES collected by the NCHS for our study. No patient was involved in the design of our study. We clearly stated that urine sample data and medical conditions questionnaires were part of the NHANES survey.

Results

Baseline Characteristics

In this study, a total of 2795 participants were included, consisting of 210 FBC participants and 2585 normal controls. Participants without urinary lead data were excluded from the study. A schematic diagram illustrating the inclusion of the participants is presented in Fig. 1. This flowchart was created using draw.io software version 22.0.3 (https://app.diagrams.net; Open Access software, The United Kingdom). The baseline characteristics of study participants are presented in Table 1, which exhibits different demographic characteristics. The urinary lead levels in the FBC population were higher than those of normal controls. FBC participants were more likely to be older (average age: 65.71 $\pm$ 12.63 *vs* 49.95 $\pm$ 17.67), Non-Hispanic White, have lower levels of affluence, and higher education (high education: 80.00% *vs* 71.49%), without a sexual partner, compared to female participants without breast cancer (BC). FBC participants were also more likely to have hypertension, diabetes, ever use female hormones, or have a smoking history. However, there were no significant differences in the distribution between participants with and without BC regarding BMI, renal function status, physical activity, and age at menarche, ever been pregnant, oral contraceptive use, and occupation classification.

Distribution of Urinary Lead Levels in the Overall Included Population and Different Subgroups

As can be seen from Table 2, the urinary lead levels increased progressively with age and BMI. Non-Hispanic Black and Mexican Americans had higher urinary lead levels than Non-Hispanic White, while other Race - Including Multi-Racial group had lower urinary lead levels than Non-Hispanic White. There were no significant differences between other Hispanic and non-Hispanic White. No significant change in urinary lead levels was found when considering the status of marital, hypertension, diabetes, renal function, physical activity, and occupation classification. However, urinary lead levels were higher in the population with a smoking history than those of non-smokers in the past years. When taking reproductive health conditions into account, the population with oral contraceptive use had lower urinary lead levels. Higher urinary lead levels were observed in the group who had been pregnant. No significant differences in urinary lead levels were found in different ages of menarche or with a different history of using female hormones.

Univariate Regression Analysis Risk Factors Associated With FBC

Table 3 presents a univariate binary logistic regression analysis to assess the association between baseline characteristics and FBC. The results disclosed that urinary lead levels showed a significant association with FBC. Those participants with older age, non-Hispanic White, higher education level, without a sexual partner, hypertension, diabetes history, using female hormones history, and smoking had a higher association with FBC. In contrast, family income to poverty ratio, renal function, physical activities, pregnancy history, oral contraceptive use, age at menarche, and occupation classification did not influence the association between urinary lead and FBC.

Independent Association between Urinary Lead and FBC by Multiple Linear Regression Equation

A multiple regression model was used to investigate the independent effects of urinary lead on FBC. The analyzed data, with and without adjustment, are shown in Table 4. Urinary lead level is a continuous variable, but it can also be regarded as a categorical variable. When considered as a continuous variable, increasing urinary lead levels were positively linked with FBC in the unadjusted model (OR = 2.22, 95% confidence interval (CI): 1.36–3.64, $p < 0.001$). In the adjustment model, which included age at the interview, educational status, race/ethnicity, marital status, hypertension status, diabetes status, oral contraceptive use, smoking history, BMI, and use of female hormone etc., the results of logistic regression did not alter significantly (OR = 2.16, 95% CI: 1.18–3.95, $p < 0.05$). The same trends were observed when urinary lead was recognized as a categorical variable (quartiles). In the unadjusted model, the OR of breast cancer in quartile 4 ($\geq$0.60 µg/L group) was 1.55 (95% CI: 1.02–2.36) compared with that in quartile 1 (<0.19 µg/L group) (*p* for trend <0.01). Likewise, in the fully adjusted model, participants in quartile 4 showed a higher association compared to those in quartile 1 (*p* for trend = 0.021).

Subgroup Analysis Stratified by Covariates of Clinical Importance

The correlation between urinary lead and FBC was analyzed by stratification using various covariates, including age, race/ethnicity, BMI, education status, marital status, family income to poverty ratio, hypertension status, diabetes status, renal function status, pregnant history, oral contraceptive use, smoking history, and occupation classi-

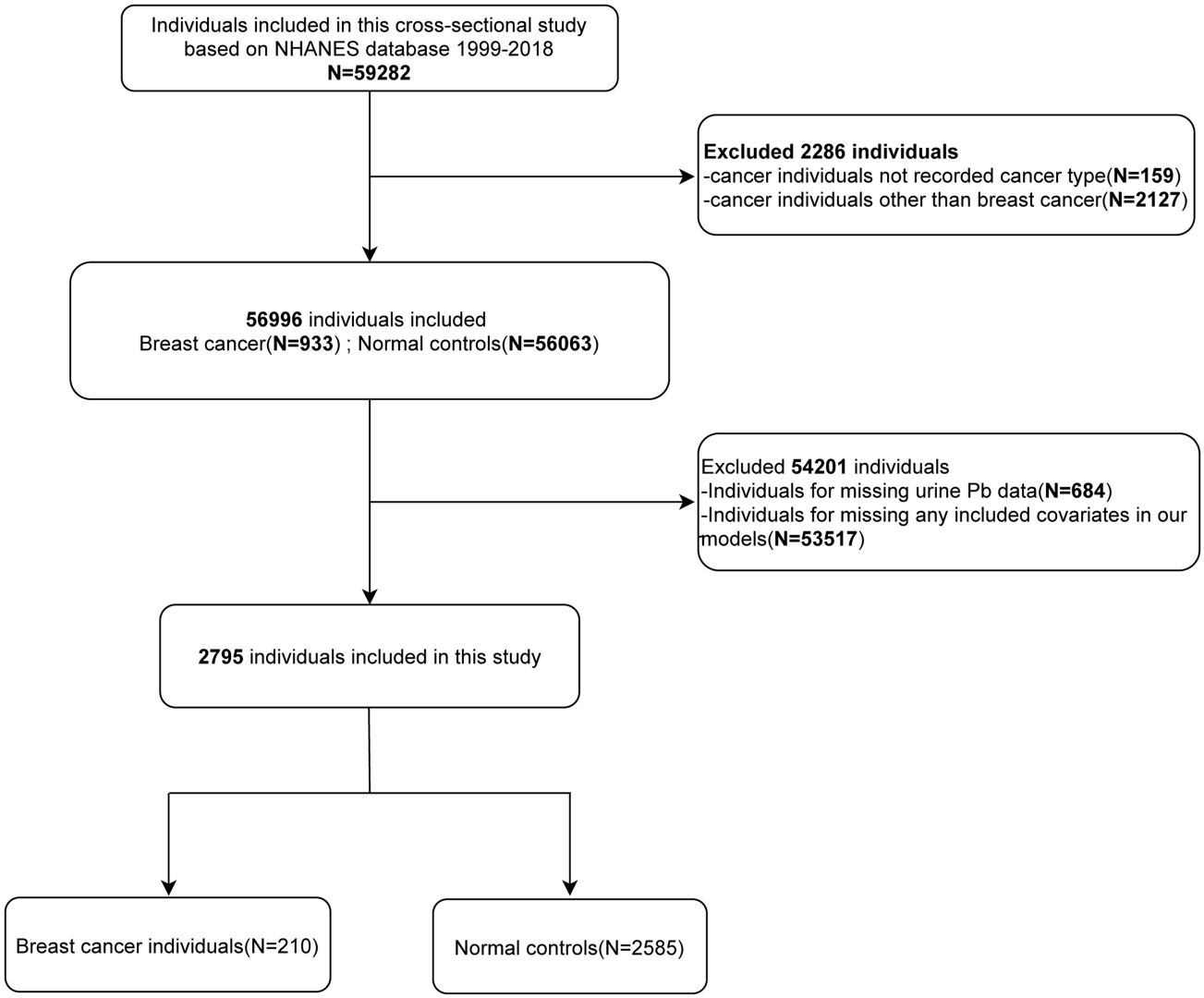

Fig. 1. A schematic diagram illustrating the inclusion of the participants. NHANES, National Health and Nutrition Examination Survey.

fication. We did not find a significant effect modification on the association between urinary lead and FBC by various subgroups (p-interaction > 0.05) except for age in the menarche subgroup (p-interaction = 0.014). This suggests that differences in age at menarche may influence our assessment of the association between urinary lead levels and FBC (Fig. 2).

Discussion

BC is a multistep process, and its pathogenesis has not been fully understood [18]. However, increasing evidence suggests that there is a strong link between the levels of serum and urinary lead and BC [17,19]. Our study firstly revealed the positive significant correlation between urinary lead and FBC, after adjusting for potential confounders among US women enrolled from NHANES 1999–2018.

The International Agency for Research on Cancer has classified lead as a carcinogen to humans (Group 2A) [20]. Although lead was previously considered a metalloestrogen that can activate the estrogen receptor in the absence of estradiol, this does not mean that lead has different expressions in different subtypes of breast cancer. Jacob K. [21] reported that lead was not associated with great odds ratios of ER/PR-negative breast cancer patients. However, evidence from SEER research indicated that lead emissions could be associated with triple-negative breast cancer, ER-positive, and PR-negative breast cancer. These conclusions are not consistent [22]. It has not been reported whether serological or urinary lead is related to breast cancer subtypes. These new topics are worth exploring in the future.

Exposure to lead may increase the risk of developing BC [23]. Previous studies have rarely reported the correlation between urinary lead and FBC, and the conclusions have not been consistent. In a case-control study, Jane A. McElroy *et al*. [16] reported that urinary lead exposure was not associated with a significantly increased risk for BC.

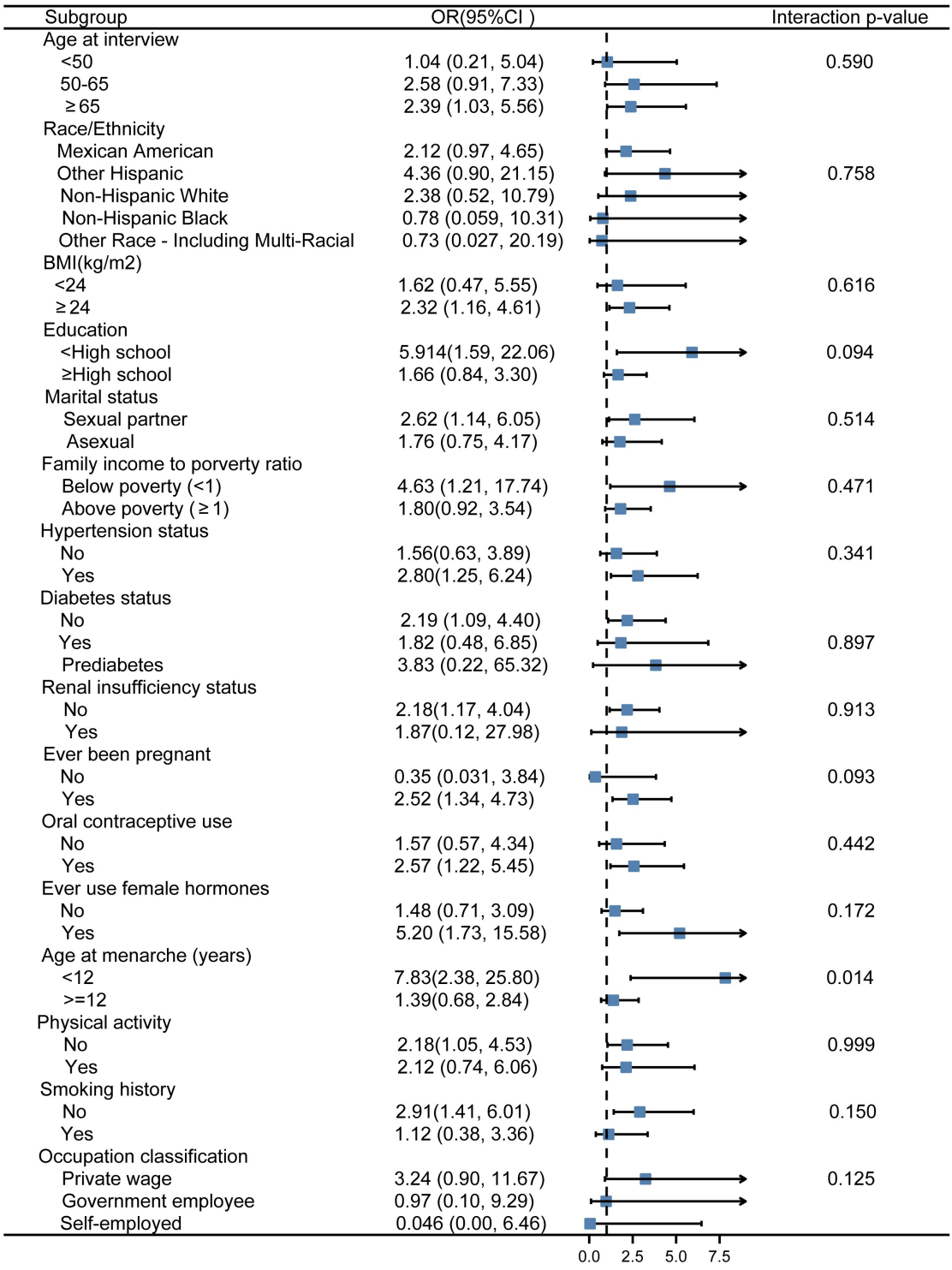

Fig. 2. Interaction effect of urinary lead on female breast cancer (FBC) in different subgroups after adjusting for confounders. Adjusting for age at interview, body mass index (BMI), race/ethnicity, marital status, education, family income to poverty ratio, hypertension status, diabetes status, kidney function status, physical activity, age at menarche, ever been pregnant, oral contraceptive use, ever use female hormones, smoking history and occupation classification except the subgroup variable. OR, Odds ratio; CI, confidence interval.

However, a strong association of urinary lead with breast cancer was observed, which was identified as a potential BC biomarker [17]. The distinct findings on the correlation between urinary lead and BC from the two studies are probably due to differences in study design, geographic regions, race/ethnicity, and control for confounding factors.

Table 1. Characteristics among female population ≥20 years of age from NHANES 1999–2018 (n = 2795).

Characteristics	Without breast cancer (n = 2585)	With breast cancer (n = 210)	t/W value	χ^2 value	*p*-value
Age at interview	49.95 ± 17.67	65.71 ± 12.63	–12.666	-	<0.001
BMI (kg/m^2)	30.22 ± 7.78	29.18 ± 6.71	1.831	-	0.067
Lead	0.37 (0.02–1.70)	0.48 (0.05–1.69)	–2.521	-	0.012
Race/Ethnicity			-	70.403	<0.001
Mexican American	525 (20.31%)	26 (12.38%)			
Other Hispanic	249 (9.63%)	15 (7.14%)			
Non-Hispanic White	841 (32.53%)	127 (60.48%)			
Non-Hispanic Black	628 (24.29%)	34 (16.19%)			
Other Race - Including Multi-Racial	342 (13.23%)	8 (3.81%)			
Marital status			-	59.316	<0.001
Asexual	1094 (42.32%)	114 (54.29%)			
Sexual partner	1491 (57.68%)	96 (45.71%)			
Education			-	13.892	0.008
<High school	737 (28.51%)	42 (20.00%)			
≥High school	1848 (71.49%)	168 (80.00%)			
Family income to poverty ratio			-	212.989	<0.001
Below poverty (<1)	611 (23.64%)	35 (16.67%)			
Above poverty (≥1)	1974 (76.36%)	153 (72.86%)			
Not recorded	0 (0.00%)	22 (10.48%)			
Hypertension status			-	26.876	<0.001
No	1625 (62.86%)	94 (44.76%)			
Yes	960 (37.14%)	116 (55.24%)			
Diabetes status			-	9.143	0.010
No	2177 (84.22%)	160 (76.19%)			
Yes	349 (13.50%)	43 (20.48%)			
Prediabetes	59 (2.28%)	7 (3.33%)			
Renal insufficiency status			-	1.938	0.164
No	2507 (96.98%)	200 (95.24%)			
Yes	78 (3.02%)	10 (4.76%)			
Physical activity			-	0.283	0.595
No	1680 (64.99%)	142 (67.62%)			
Yes	905 (35.01%)	68 (32.38%)			
Age at menarche (years)			-	2.629	0.105
<12	517 (20.00%)	48 (24.87%)			
≥12	2068 (80.00%)	145 (75.13%)			
Ever been pregnant			-	1.027	0.311
No	328 (12.69%)	20 (9.52%)			
Yes	2257 (87.31%)	176 (83.81%)			
Not recorded	0 (0.00%)	14 (6.67%)			
Oral contraceptive use			-	0.003	0.960
No	1024 (39.61%)	78 (37.14%)			
Yes	1561 (60.39%)	118 (56.19%)			
Not recorded	0 (0.00%)	14 (6.67%)			
Ever use female hormones			-	40.609	<0.001
No	2108 (81.55%)	136 (64.76%)			
Yes	477 (18.45%)	57 (27.14%)			
Not recorded	0 (0.00%)	17 (8.10%)			
Smoking history			-	22.629	<0.001
No	1990 (76.98%)	131 (62.38%)			
Yes	595 (23.02%)	79 (37.62%)			

Table 1. Continued.

Characteristics	Without breast cancer (n = 2585)	With breast cancer (n = 210)	t/W value	χ^2 value	*p*-value
Occupation classification			-	2.397	0.302
Private wage	895 (34.6%)	37 (17.6%)			
Government employed	249 (9.6%)	14 (6.7%)			
Self-employed	111 (4.3%)	8 (3.8%)			
Not recorded	1330 (51.5%)	151 (71.9%)			

BMI, body mass index.

Table 2. Urinary lead levels in the total study population and subgroups by selected variables.

Characteristics	n	Urinary lead levels (µg/L)	W value	*p* value
Total population	2795	0.40 (0.21, 0.72)		
Age at interview				
<50	1325	0.36 (0.18, 0.65)	Reference	
50~65	741	0.42 (0.22, 0.76) *	3.7480	<0.001
≥65	729	0.46 (0.25, 0.82) *	5.8130	<0.001
BMI (kg/m^2)				
<24	592	0.34 (0.18, 0.61)	Reference	
≥24	2194	0.41 (0.22, 0.77) *	4.749	<0.001
Race/ethnicity				
Non-Hispanic White	968	0.36 (0.20, 0.63)	Reference	
Non-Hispanic Black	662	0.50 (0.26, 0.84) *	6.3740	<0.001
Mexican American	551	0.49 (0.24, 0.90) *	5.6290	<0.001
Other Hispanic	264	0.34 (0.19, 0.60)	–0.6780	0.960
Other Race - Including Multi-Racial	350	0.29 (0.15, 0.51) *	–3.9220	<0.001
Marital status				
Sexual partner	1587	0.39 (0.20, 0.70)	Reference	
Asexual	1208	0.41 (0.22, 0.76)	1.6820	0.093
Education				
<High school	779	0.50 (0.26, 0.87)	Reference	
≥High school	2016	0.37 (0.20, 0.66) *	–6.9020	<0.001
Family income to poverty ratio				
Below poverty (<1)	646	0.42 (0.23, 0.79)	Reference	
Above poverty (≥1)	2127	0.39 (0.20, 0.71) *	–2.6360	0.009
Not recorded	22	0.64 (0.35, 0.94)	-	-
Hypertension status				
No	1719	0.40 (0.20, 0.71)	Reference	
Yes	1076	0.40 (0.22, 0.74)	–1.0689	0.285
Diabetes status				
No	2337	0.40 (0.21, 0.72)	Reference	
Yes	392	0.40 (0.21, 0.73)	0.1480	0.987
Prediabetes	66	0.41 (0.27, 0.72)	1.0960	0.501
Renal insufficiency status				
No	2707	0.40 (0.21, 0.72)	Reference	
Yes	88	0.30 (0.17, 0.66)	1.3296	0.187
Physical activity				
No	1817	0.40 (0.21, 0.74)	Reference	
Yes	973	0.39 (0.20, 0.70)	–1.4270	0.154
Not recorded	5	0.55 (0.42, 2.30)	-	-
Age at menarche (years)				
<12	565	0.42 (0.22, 0.78)	Reference	
≥12	2213	0.40 (0.21, 0.71)	–1.5420	0.124
Not recorded	17	0.49 (0.38, 0.91)	-	-

Table 2. Continued.

Characteristics	n	Urinary lead levels (µg/L)	W value	*p* value
Ever been pregnant				
No	348	0.30 (0.17, 0.54)	Reference	
Yes	2433	0.41 (0.22, 0.76) *	6.0160	<0.001
Not recorded	14	-	-	-
Oral contraceptive use				
No	1102	0.42 (0.22, 0.80)	Reference	
Yes	1679	0.38 (0.20, 0.70) *	–2.7460	0.006
Not recorded	14	-	-	-
Ever use female hormones				
No	2244	0.39 (0.20, 0.72)	Reference	
Yes	534	0.42 (0.23, 0.73)	1.9970	0.050
Not recorded	2	-	-	-
Smoking history				
No	2121	0.39 (0.20, 0.70)	Reference	
Yes	674	0.44 (0.23, 0.83) *	–3.0384	0.002
Occupation classification				
Private wage	932 (33.35%)	0.32 (0.18, 0.55)	Reference	
Government employed	263 (9.41%)	0.36 (0.18, 0.61)	0.945	0.605
Self-employed	119 (4.26%)	0.35 (0.19, 0.57)	1.111	0.500
Not recorded	1481 (52.99%)	0.37 (0.20, 0.61)	-	-

* Statistically significant difference compared to the reference group.

Table 3. Crude association between urinary lead and FBC in baseline characteristic.

Characteristics	β	SE	Wald χ^2	No. (%)	OR (95% CI)	*p* value
Age at interview						
<50	0.00			1325 (47.41%)	1.0	
≥50, <65	1.48	0.23	40.70	741 (26.51%)	4.38 (2.78, 6.89)	<0.001
≥65	2.19	0.22	103.02	729 (26.08%)	8.95 (5.86, 13.66)	<0.001
BMI (kg/m^2)						
<24	0.00			592 (21.25%)	1.0	
≥24	–0.16	0.17	0.89	2194 (78.75%)	0.85 (0.61, 1.19)	0.344
Lead	0.80	0.25	10.11	2521	2.22 (1.36, 3.64)	<0.001
Race/ethnicity						
Non-Hispanic White	0.00			968 (34.63%)	1.0	
Non-Hispanic Black	–1.02	0.20	26.26	662 (23.69%)	0.36 (0.24, 0.53)	<0.001
Mexican American	–1.11	0.22	25.15	551 (19.71%)	0.33 (0.21, 0.51)	<0.001
Other Hispanic	–0.92	0.28	10.59	264 (9.45%)	0.40 (0.23, 0.69)	0.001
Other Race - Including Multi-Racial	–1.86	0.37	25.39	350 (12.52%)	0.15 (0.07, 0.32)	<0.001
Marital status						
Sexual partner	0.00			1587 (56.78%)	1.0	
Asexual	0.48	0.14	11.16	1208 (43.22%)	1.62 (1.22, 2.15)	<0.001
Education						
<High school	0.00			779 (27.87%)	1.0	
≥High school	0.47	0.18	6.89	2016 (72.13%)	1.60 (1.13, 2.26)	0.009
Family income to poverty ratio						
<1	0.00			646 (23.11%)	1.0	
≥1	0.30	0.19	2.45	2127 (76.10%)	1.35 (0.93, 1.98)	0.117
Hypertension status						
No				1719 (61.50%)	1.0	
Yes	0.74	0.14	25.94	1076 (38.50%)	2.09 (1.57, 2.77)	<0.001

Table 3. Continued.

Characteristics	β	SE	Wald χ^2	No. (%)	OR (95% CI)	p value
Diabetes status						
No	0.00			2337 (83.61%)	1.0	
Yes	0.52	0.18	8.13	392 (14.03%)	1.68 (1.18, 2.39)	0.004
Prediabetes	0.48	0.41	1.38	66 (2.36%)	1.61 (0.73, 3.59)	0.241
Renal inefficiency status						
No	0.00			2707 (96.85%)	1.0	
Yes	0.47	0.34	1.90	88 (3.15%)	1.61 (0.82, 3.15)	0.168
Physical activity						
No	0.00			1817 (65.01%)	1.0	
Yes	–0.082	0.15	0.28	973 (34.81%)	0.92 (0.68, 1.25)	0.595
Age at menarche (years)						
<12	0.00			565 (20.21%)	1.0	
≥ 12	–0.28	0.17	2.61	2213 (79.18%)	0.76 (0.54, 1.06)	0.106
Ever been pregnant						
No	0.00			348 (12.51%)	1.0	
Yes	0.25	0.24	1.02	2433 (87.49%)	1.28 (0.79, 2.06)	0.312
Oral contraceptive use						
No	0.00			1102 (39.63%)	1.0	
Yes	–0.0076	0.15	0.0025	1679 (60.37%)	0.99 (0.74, 1.34)	0.960
Ever use female hormones						
No	0.00			2244 (80.72%)	1.0	
Yes	0.62	0.17	13.83	534 (19.21%)	1.85 (1.34, 2.56)	<0.001
Smoking history						
No	0.00			2121 (75.89%)	1.0	
Yes	0.70	0.15	21.90	674 (24.11%)	2.02 (1.50, 2.71)	<0.001
Occupation classification						
Private wage				932 (33.35%)	1.0	
Government employed	0.31	0.32	0.91	263 (9.41%)	1.36 (0.72, 2.56)	0.339
Self-employed	0.56	0.40	1.91	119 (4.26%)	1.74 (0.79, 3.84)	0.168

Table 4. The association of urinary lead levels with FBC in the cross-sectional study from NHANES 2009–2018.

Outcome	Model I				Model II				Model III			
	β	SE	Wald χ^2	OR (95% CI)	β	SE	Wald χ^2	OR (95% CI)	β	SE	Wald χ^2	OR (95% CI)
Urinary Pb, per 1 mg/L increase	0.80	0.25	10.11	2.22 (1.36, 3.64) ***	0.63	0.27	28.52	1.87 (1.10, 3.18) *	0.74	0.29	41.60	2.16 (1.18, 3.95) *
Pb levels Quartiles												
Q1 (<0.19)			Reference				Reference				Reference	
Q2 (≥0.19–<0.35)	–0.20	0.24	0.67	0.82 (0.51, 1.32)	–0.37	0.26	2.10	0.69 (0.42, 1.14)	–0.25	0.28	0.82	0.78 (0.45, 1.35)
Q3 (≥0.35–<0.60)	0.19	0.22	0.70	1.21 (0.78, 1.87)	0	0.24	0	1.00 (0.63, 1.60)	0.0079	0.28	0.00082	1.01 (0.59, 1.73)
Q4 (≥0.60)	0.44	0.21	4.21	1.55 (1.02, 2.36) *	0.26	0.23	1.32	1.30 (0.83, 2.03)	0.39	0.26	2.27	1.48 (0.89, 2.48)
p value for trend			<0.010				0.032				0.021	

Note: *, $p < 0.05$, ***, $p < 0.001$. Model I: no covariates were adjusted. Model II: Age at interview and BMI (kg/m^2) were adjusted. Model III: Age at interview, BMI, education, race/ethnicity, marital status, family income to income to poverty ratio, hypertension status, diabetes status, renal insufficiency status; physical activity, ever been pregnant, oral contraceptive use, ever use female hormones, age at menarche, smoking history, and occupation classification were adjused. Q means quartile.

Our cross-sectional case-control study, which is different from previous case-control designs, further disclosed the independent association between urinary lead and FBC in a large-scale epidemiological survey. The urinary lead levels were divided into quartiles to understand the relationship between urinary lead exposure and FBC. Compared with the lowest urinary lead group (Q1), the association between urinary lead and FBC increased by 21% in the medium urinary lead (Q3) and 55% in the highest urinary lead (Q4) in the unadjusted model (no covariates were adjusted; p for trend <0.01). Similarly, the fully adjusted model followed the same trend (all the potential confounding factors were adjusted, p for trend = 0.021). Therefore, as demonstrated, our findings are robust and urinary lead exposure could be an independent association with FBC.

The exact mechanism underlying lead exposure in BC is not yet fully understood. Lead is one of the bivalent cationic metalloestrogens that activates the genomic and nongenomic pathways of Erα, inducing the proliferation of estrogen-dependent breast cancer cells. This supports the estrogen-like effects of these bivalent cationic metals *in vitro* [10,24]. Heavy metals are considered the most important environmental contaminants and potential risks to BC [23,25]. Exposure to lead pollutants has been associated with postmenopausal breast cancer [9]. Consistent with previous studies [25], urinary lead levels were elevated with aging or BMI rising, indicating that both elements were FBC risks. Urinary lead levels were higher in Mexican Americans and Non-Hispanic Black populations compared to Non-Hispanic White and other races. In addition, urinary lead levels in populations with higher education levels or above poverty were lower than those with lower education and below poverty. Urinary lead levels were weakly lower in the population when they had taken oral contraceptive medications. However, we did not find baseline differences in urinary lead levels in different statuses of blood pressure, renal function, diabetes, reproductive health conditions, and smoking history. Taken together, we speculate that changes in urinary lead levels are influenced by some potential confounders.

This current study has several strengths. First, this study combined the ten waves of the latest NHANES database. Second, the data provide, for the first time, the exact positive association between urinary lead and FBC as estimated by a cross-sectional case-control study. Third, certain potential confounding factors (socio-demography, reproductive health conditions, diabetes, renal function, blood pressure, and physical activity) were incorporated into this study. Finally, this research is innovative to some extent because few studies have been reported on the relationship between urinary lead and FBC.

There are some limitations to consider regarding the conclusion of the positive association between urinary lead and FBC. These limitations include geographic limitations, as the study is based on NHANES data, which was conducted only in the United States. It is unknown whether this conclusion can be extended to other regions and populations. Second, this present cross-sectional case-control study could not interpret the causality between urinary lead and FBC. Third, even though a comprehensive set of confounders was considered, residual or unmeasured confounding factors (such as dietary status) may still exist. Fourth, the NHANES questionnaire is self-reported, which could not provide the detailed pathological stage and disease subtypes of the breast cancer population. Thus, the data may cause a certain degree of social desirability or bias. Additionally, previous reports indicated that bone lead accounts for more than 90% of the lead load of the adult body [26]. The serum lead reflects the body's recent lead exposure, and the urinary lead only reflects the part of serum secreted through the kidney, neither of which can reflect the actual lead load in the body [16,27,28]. Therefore, further in-depth studies are required to understand the exact effect of lead exposure on the mechanism and etiology of FBC.

Conclusions

This cross-sectional case-control study found that urinary lead levels were significantly higher in FBC participants compared to healthy controls. The increasing urinary lead levels are likely independently associated with FBC. However, further research is necessary to substantiate the real molecular mechanism and association between urinary lead and FBC.

Abbreviations

BC, breast cancer; FBC, female breast cancer; NHANES, National Health and Nutrition Examination Survey; OR, Odds ratio; CI, confidence interval; NCHS, National Center for Health Statistics; ICP-MS, Inductively Coupled Plasma-Mass Spectrometry; BMI, body mass index.

Availability of Data and Materials

All the data sets used are freely available from theNHANES website public archive, accessible at NHANES Questionnaires, Data sets and Related Documentation repository [https://wwwn.cdc.gov/nchs/nhanes/Default.aspx].

Author Contributions

DH and LZ collectively accomplished writing of this manuscript. DH participated acquision of data. LZ participated the data collection and accounted for interpretation of data. NW prepared the Figs. 1,2 inspected all the results and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and

resolved. BQ revised the manuscrip critically for important intellectual content and gave the final approval of the version to be published. DH and BQ made all the tables, responsible for the accuracy or integrity of their work part. And NW and BQ both participated in data analysis of this study. WS and XL have provided this study design, analysis, interpretation of study and overall supervision. All authors reviewed the manuscript. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Informed consent was provided by all participants of the NHANES study, more information is available here: https://www.cdc.gov/nchs/nhanes/hlthprofess.htm (accessed on 10 August 2023). The NHANES study protocol was approved by the research ethics review board of the National Center for Health Statistics (NCHS), and all participants provided written informed consent. Informed consent was obtained from all subjects and/or their legal guardian(s). NCHS Ethics Review Board (ERB) Approval could be searched in https://www.cdc.gov/nchs/nhanes/irba98.htm#print.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Obeng-Gyasi E. Sources of lead exposure in various countries. Reviews on Environmental Health. 2019; 34: 25–34.

[2] Pottier G, Viau M, Ricoul M, Shim G, Bellamy M, Cuceu C, *et al*. Lead Exposure Induces Telomere Instability in Human Cells. PLoS ONE. 2013; 8: e67501.

[3] Gundacker C, Forsthuber M, Szigeti T, Kakucs R, Mustieles V, Fernandez MF, *et al*. Lead (Pb) and neurodevelopment: A review on exposure and biomarkers of effect (BDNF, HDL) and susceptibility. International Journal of Hygiene and Environmental Health. 2021; 238: 113855.

[4] Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metal toxicity and the environment. Experientia Supplementum. 2012; 101: 133–164.

[5] Pizzol M, Thomsen M, Andersen MS. Long-term human exposure to lead from different media and intake pathways. The Science of the Total Environment. 2010; 408: 5478–5488.

[6] Rai PK, Lee SS, Zhang M, Tsang YF, Kim KH. Heavy metals in food crops: Health risks, fate, mechanisms, and management. Environment International. 2019; 125: 365–385.

[7] Ding N, Wang X, Tucker KL, Weisskopf MG, Sparrow D, Hu H, *et al*. Dietary patterns, bone lead and incident coronary heart disease among middle-aged to elderly men. Environmental Research. 2019; 168: 222–229.

[8] Satarug S, C Gobe G, A Vesey D, Phelps KR. Cadmium and Lead Exposure, Nephrotoxicity, and Mortality. Toxics. 2020; 8: 86.

[9] White AJ, O'Brien KM, Niehoff NM, Carroll R, Sandler DP. Metallic Air Pollutants and Breast Cancer Risk in a Nationwide Cohort Study. Epidemiology. 2019; 30: 20–28.

[10] Martin MB, Reiter R, Pham T, Avellanet YR, Camara J, Lahm M, *et al*. Estrogen-like activity of metals in MCF-7 breast cancer cells. Endocrinology. 2003; 144: 2425–2436.

[11] Tapia-Orozco N, Santiago-Toledo G, Barrón V, Espinosa-García AM, García-García JA, García-Arrazola R. Environmental epigenomics: Current approaches to assess epigenetic effects of endocrine disrupting compounds (EDC's) on human health. Environmental Toxicology and Pharmacology. 2017; 51: 94–99.

[12] Mansouri B, Ramezani Z, Yousefinejad V, Nakhaee S, Azadi N, Khaledi P, *et al*. Association between trace elements in cancerous and non-cancerous tissues with the risk of breast cancers in western Iran. Environmental Science and Pollution Research International. 2022; 29: 11675–11684.

[13] Rzymski P, Tomczyk K, Rzymski P, Poniedziałek B, Opala T, Wilczak M. Impact of heavy metals on the female reproductive system. Annals of Agricultural and Environmental Medicine. 2015; 22: 259–264.

[14] Panaiyadiyan S, Quadri JA, Nayak B, Pandit S, Singh P, Seth A, *et al*. Association of heavy metals and trace elements in renal cell carcinoma: A case-controlled study. Urologic Oncology. 2022; 40: 111.e11–111.e18.

[15] Wu L, Cui F, Zhang S, Ding X, Gao W, Chen L, *et al*. Associations between multiple heavy metals exposure and neural damage biomarkers in welders: A cross-sectional study. The Science of the Total Environment. 2023; 869: 161812.

[16] McElroy JA, Shafer MM, Gangnon RE, Crouch LA, Newcomb PA. Urinary lead exposure and breast cancer risk in a population-based case-control study. Cancer Epidemiology, Biomarkers & Prevention. 2008; 17: 2311–2317.

[17] Burton C, Dan Y, Donovan A, Liu K, Shi H, Ma Y, *et al*. Urinary metallomics as a novel biomarker discovery platform: Breast cancer as a case study. Clinica Chimica Acta. 2016; 452: 142–148.

[18] Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY, *et al*. Risk Factors and Preventions of Breast Cancer. International Journal of Biological Sciences. 2017; 13: 1387–1397.

[19] Kilkkinen A, Virtamo J, Vartiainen E, Sankila R, Virtanen MJ, Adlercreutz H, *et al*. Serum enterolactone concentration is not associated with breast cancer risk in a nested case-control study. International Journal of Cancer. 2004; 108: 277–280.

[20] Gaudet MM, Deubler EL, Kelly RS, Ryan Diver W, Teras LR, Hodge JM, *et al*. Blood levels of cadmium and lead in relation to breast cancer risk in three prospective cohorts. International Journal of Cancer. 2019; 144: 1010–1016.

[21] Kresovich JK, Erdal S, Chen HY, Gann PH, Argos M, Rauscher GH. Metallic air pollutants and breast cancer heterogeneity. Environmental Research. 2019; 177: 108639.

[22] Ro E, Vu V, Wei Y. Ambient air emissions of endocrine-disrupting metals and the incidence of hormone receptor- and HER2-dependent female breast cancer in USA. Medical Oncology. 2022; 39: 69.

[23] Byrne C, Divekar SD, Storchan GB, Parodi DA, Martin MB. Metals and breast cancer. Journal of Mammary Gland Biology and Neoplasia. 2013; 18: 63–73.

[24] Choe SY, Kim SJ, Kim HG, Lee JH, Choi Y, Lee H, *et al*. Evaluation of estrogenicity of major heavy metals. The Science of the Total Environment. 2003; 312: 15–21.

[25] Alatise OI, Schrauzer GN. Lead exposure: a contributing cause of the current breast cancer epidemic in Nigerian women. Biological Trace Element Research. 2010; 136: 127–139.

[26] Specht AJ, Lin Y, Weisskopf M, Yan C, Hu H, Xu J, *et al*. XRF-measured bone lead (Pb) as a biomarker for Pb exposure and toxicity among children diagnosed with Pb poisoning. Biomarkers. 2016; 21: 347–352.

[27] Nieboer E, Tsuji LJS, Martin ID, Liberda EN. Human biomonitoring issues related to lead exposure. Environmental Science. Processes & Impacts. 2013; 15: 1824–1829.

[28] Wilker E, Korrick S, Nie LH, Sparrow D, Vokonas P, Coull B, *et al*. Longitudinal changes in bone lead levels: the VA Normative Aging Study. Journal of Occupational and Environmental Medicine. 2011; 53: 850–855.