Urinary Cadmium and Serum Levels of Estrogens and Androgens in Postmenopausal Japanese Women

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Abstract

Background: Recent laboratory studies have suggested that cadmium is an estrogenic compound and may be a potential risk factor for breast cancer.

Methods: We investigated the relationship between urinary cadmium concentrations and serum concentrations of estrone, testosterone, and dehydroepiandrosterone sulfate in 161 postmenopausal Japanese women.

Results: There was a significant positive association between the urinary cadmium and serum testosterone levels after controlling for age and body mass index. The mean testosterone level was 28% higher in women with high urinary cadmium (>2.00 μg/g creatinine) than in those with low urinary cadmium (<2.00 μg/g creatinine). Urinary cadmium was not significantly associated with serum estrone and dehydroepiandrosterone sulfate levels. Additional adjustment for smoking, alcohol and reproductive factors including known risk factors for breast cancer did not substantially alter the results.

Conclusion: Data suggested that cadmium exposure is associated with increased testosterone levels. As high testosterone levels have been associated with the risk of breast cancer, the involvement of cadmium exposure in breast cancer risk should be evaluated in future studies.

(Cancer Epidemiol Biomarkers Prev 2005;14(3):705–8)

Introduction

Cadmium is a highly toxic metal for humans, well known for its occupational health risks. The main source of nonoccupational exposure to cadmium in the general population includes smoking, air, and food and water contaminated by cadmium (1). An abnormal amount of cadmium in the body has been associated with a reproductive hazard by a mechanism possibly resulting from direct insult on the reproductive organs such as degeneration, necrosis, inflammation, edema, etc. (1). However, it was recently reported that cadmium may have estrogenicity (2-4). Stoica et al. (2) found that at lower concentrations, cadmium competes with estradiol for binding to estrogen receptors and activates the receptors, leading to changes in gene expression. Although the mechanism for estrogenicity remains to be elucidated in further studies, they observed that cadmium acts as an estrogen in the uterus and mammary gland in rats (3). They suggested that cadmium may represent a new class of endocrine disruptors and that exposure to cadmium could be a potential risk factor for breast cancer. Some studies investigated the relationship between exposure to cadmium and endogenous reproductive hormone levels in men (5-7), but data for women is scanty. The association between cadmium exposure and endogenous reproductive hormone levels in women is of interest in light of the fact that estradiol for binding to estrogen receptors and activates the estrogen receptors, leading to changes in gene expression. Although the mechanism for estrogenicity remains to be elucidated in further studies, they observed that cadmium acts as an estrogen in the uterus and mammary gland in rats (3). They suggested that cadmium may represent a new class of endocrine disruptors and that exposure to cadmium could be a potential risk factor for breast cancer. Some studies investigated the relationship between exposure to cadmium and endogenous reproductive hormone levels in men (5-7), but data for women is scanty. The association between cadmium exposure and endogenous reproductive hormone levels in women is of interest in light of the fact that increased reproductive hormone levels have been associated with a risk of breast cancer in women (8). The urinary excretion of cadmium is proportional to the body burden and is widely used as a dosimeter of lifetime exposure to cadmium (9). In the present study, we examined the association between urinary cadmium level and serum hormone levels of estrone, estradiol, testosterone, and dehydroepiandrosterone sulfate (DHEAS) in postmenopausal Japanese women.

Materials and Methods

Between 2000 and 2002, women attending a breast cancer screening at a general hospital in Gifu, Japan, were recruited for a study of mammographic breast density. About 50% of the participants in the screening were annual attendants. The main purpose of the study was to identify the determinants of mammographic breast density. The number of participants in the screening were 656 in 2000, 924 in 2001, and 838 in 2002; among them, 461, 613, and 356 persons participated in the study, respectively. Therefore, response rates were 70.3% in 2000, 66.3% in 2001, and 42.5% in 2002. The response rates were low in 2001 and 2002. Some women who previously participated in the screening, or both the screening and the study of this project, chose to participate only in the screening, but not in the study of the subsequent year, therefore causing the subsequent reduction in the response rates. Hence, the response rate (70.3%) in 2000 represents the responses among those newly visited the screening during the study period. Altogether, a total of 1,072 individuals, 1,430 persons including the number of individuals who revisited the study, participated in the study. Blood and urine samples were collected from each subject. The study also aimed to examine the associations of various biomarkers with breast cancer risk factors as well as the risk of breast cancer using subsets of this population. The study period for each component study was predetermined. The present study included subpopulations for measurements of urinary cadmium and serum hormone levels.

At recruitment, women completed a self-administered questionnaire asking basic demographic characteristics, diet, physical activity, smoking and drinking habits, medical history, and reproductive history. They were also asked about menopausal status and if they were postmenopausal, type of...
menopause (natural or surgical), and age at menopause. The study was restricted to postmenopausal women without a menstrual cycle in the past 12 months. Women were excluded if they were aged ≤49 years and had surgical menopause with ovarian conservation. Informed consent was obtained from each woman. This study was approved by the Institutional Review Board.

Diet, including alcohol intake, was assessed with a validated 169-item semiquantitative food-frequency questionnaire (10). The questionnaire asked participants how often on average they consumed each of the food items listed and what the usual serving size of each item was during the year prior to the study. The intake of foods and nutrients were estimated by frequency of ingestion and portion size using the Japanese Standard Tables of Food Composition, 4th and 5th editions, published by the Science and Technology Agency of Japan (11). Detailed information on the questionnaire, including its validity and reproducibility, has been described elsewhere (10, 12). For example, the Spearman correlation coefficients between this questionnaire and 12 daily diet records kept over a 1-year period for intake of alcohol, calcium, copper, zinc, and iron ranged from 0.51 to 0.73.

Blood and urine samples were obtained from the subjects at about 2:00 p.m. Urine was frozen at –80°C until analysis. Urinary cadmium was determined by flameless atomic absorption spectrometry (Z-5700 model, Hitachi, Tokyo; ref. 13). A total of 15 (9.1%) women had urinary cadmium below sensitivity levels; thus, they were assigned the values of the assay sensitivity (= 0.4 μg/L). The intra- and interassay coefficients of variation were 4.3% and 6.7%, respectively. To adjust for variation in the diluteness of urine, urinary cadmium levels were expressed as urine cadmium / urine creatinine. The sera were separated after centrifugation and stored at –80°C until assayed. Serum estrone was measured by RIA using kits purchased from the Diagnostic Systems Corporation, Los Angeles, CA. The sensitivity and the interassay coefficient of variation, 1.4 μg/mL and <13.7% for estradiol; 4 ng/dL and 12.3% to 23.9% for high to low levels of testosterone; and 1.1 ng/mL and <12.8% for DHEAS, respectively.

Urine and serum was measured among 187 women (without duplication). However, we excluded 21 women from analyses because of incomplete or unreliable responses to the dietary questionnaire (criteria shown in ref. 10). Additionally, we further excluded two women because their estradiol levels suggested unreported estrogen use (>100 pg/mL). The remaining 164 women are the focus of this report. DHEAS was not measured in two women because of insufficient volume for the measurement. Of these women, 91 (55.5%) had estradiol levels below the sensitivity of the assay. Thus, the analysis for estradiol was eliminated from this study. Twenty-two (13.4%) women had estrone levels, and 27 (16.5%) had testosterone levels below the sensitivity of the assay; the values of the assay sensitivity were assigned for them.

Urinary cadmium and hormone levels were transformed into logarithmic values for statistical analysis. The relationships between urinary cadmium and hormone levels were assessed by linear regression models. We used analysis of covariance method to provide adjusted estimates of the geometric means of hormone levels according to the level of urinary cadmium. Age and body mass index (BMI) were always included in the models as covariates. It is known that one of the major sources of cadmium exposure is cigarette smoking (1). However, most study subjects were never-smokers. Smoking status and other factors including parity, age at menarche, age at first birth, age at menopause, family history of breast cancer among the relatives of first degree and intakes of macro- and micronutrients were examined as potential confounders by including them into the models. Nutrient intakes were logarithmically transformed. All statistical analyses were done using SAS.

Results

Table 1 presents the characteristics of 164 women. Most of the women were never-smokers: the number (%) of current, ex-, and never-smokers were 8 (4.9%), 5 (3.0%), and 148 (90.2%), respectively. Smoking status was missing for three women. The geometric mean of urinary cadmium concentration in 164 women was 2.4 μg/g creatinine with 95% confidence interval of 2.2 to 2.5. The distribution of urinary cadmium is shown in Table 2.

Urinary cadmium was significantly associated with serum testosterone levels after controlling for age and BMI (Table 3). The mean testosterone level was 28% higher in women with high urinary cadmium (≥3.00 μg/g creatinine) than in those with low urinary cadmium (<2.00 μg/g creatinine). There was no significant association between urinary cadmium and serum levels of estrone and DHEAS.

The levels of urinary cadmium tended to be higher in current or ex-smokers than those in never-smoker (age-adjusted geometric means were 2.60, 3.22, and 2.30 μg/g creatinine for current, ex-, and never-smokers, respectively). Additional adjustment for smoking status, alcohol intake, years of education, age at menarche, parity, age at first full birth, age at menopause, and family history of breast cancer did not substantially alter the association between urinary cadmium and serum testosterone: the mean testosterone level was 31% higher in women in the highest than those in the lowest category of urinary cadmium.

Adjustment of the intake of calcium, copper, zinc, and iron, which may influence the absorption of cadmium, did not substantially alter the results (data not shown).

The reliability of measurement of testosterone was assessed in another subsample of postmenopausal women (n = 60) in which blood sampling was repeated with about 1-year intervals. The intra-crass correlation coefficient for testosterone was 0.51. Although this value was lower than those previously reported among somewhat elder white women [intra-crass correlation coefficient, 0.88 over 2 years in women ages 55-69 (ref. 14), intra-crass correlation coefficient, 0.92 over 1 year in women with a mean of 9 years since the onset of menopause (ref. 15)], hormone values might actually change within a few years after the onset of menopause. After excluding 24 women aged ≤55 in our subsample, the intra-crass correlation coefficient for testosterone was reasonably high (0.79). We also reanalyzed data in the present study after restricting the study subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>59.0 (6.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 (3.0)</td>
</tr>
<tr>
<td>Years of education (y)</td>
<td>10.9 (2.1)</td>
</tr>
<tr>
<td>Age at menarche (y)</td>
<td>14.2 (2.8)</td>
</tr>
<tr>
<td>Number of births</td>
<td>2.4 (0.8)</td>
</tr>
<tr>
<td>Age at first birth (y)</td>
<td>24.7 (2.8)</td>
</tr>
<tr>
<td>Age at menopause (y)</td>
<td>49.6 (3.9)</td>
</tr>
<tr>
<td>Total energy intake (kcal/d)</td>
<td>2,202 (676)</td>
</tr>
<tr>
<td>Alcohol intake (mL/d)</td>
<td>5.5 (15.0)</td>
</tr>
</tbody>
</table>

Table 1. Means of basic characteristics of 164 postmenopausal women

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Table 2. Distribution of urinary cadmium concentrations in 164 women

<table>
<thead>
<tr>
<th>Urinary cadmium (µg/g creatinine)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.00</td>
<td>4 (2.4)</td>
</tr>
<tr>
<td>1.00-1.99</td>
<td>45 (27.4)</td>
</tr>
<tr>
<td>2.00-2.99</td>
<td>70 (42.7)</td>
</tr>
<tr>
<td>3.00-3.99</td>
<td>29 (17.7)</td>
</tr>
<tr>
<td>4.00-5.35</td>
<td>16 (9.8)</td>
</tr>
<tr>
<td>Total</td>
<td>164 (100)</td>
</tr>
</tbody>
</table>

Table 3. Adjusted geometric means of serum hormone levels according to urinary cadmium

<table>
<thead>
<tr>
<th>Urinary cadmium (µg/g creatinine)</th>
<th>No.</th>
<th>Estrone (pmol/L)</th>
<th>Testosterone (nmol/L)</th>
<th>DHEAS (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All women</td>
<td>164</td>
<td>21.5 (18.5-24.8)</td>
<td>0.29 (0.27-0.32)</td>
<td>2,360 (2,176-2,564)</td>
</tr>
<tr>
<td>0-2.00</td>
<td>49</td>
<td>17.8 (13.3-22.9)</td>
<td>0.25 (0.21-0.29)</td>
<td>1,971 (1,700-2,287)</td>
</tr>
<tr>
<td>2.01-3.00</td>
<td>70</td>
<td>24.4 (19.6-30.7)</td>
<td>0.32 (0.28-0.37)</td>
<td>2,523 (2,228-2,852)</td>
</tr>
<tr>
<td>3.00+</td>
<td>45</td>
<td>21.5 (16.3-28.5)</td>
<td>0.32 (0.27-0.37)</td>
<td>2,592 (2,242-3,019)</td>
</tr>
</tbody>
</table>

P for trend 0.83

NOTE: Adjusted for age and BMI.

to women aged ≥55 years. The results were not substantially altered; for example, the increase in testosterone in the highest as compared with the lowest category of urinary cadmium was 35.6% after controlling for age and BMI.

Discussion

The absorbed cadmium is bound to high–molecular weight proteins in the blood and transported to the liver, where it binds to metallothionein and is then redistributed to various tissues and organs (1). After reabsorption, cadmium is accumulated in the kidney cortex. The urinary cadmium concentration is mainly influenced by the body burden and urinary cadmium is proportional to the concentration in the kidney (9). The reported mean urinary cadmium in 378 nonsmoking women from 19 regions in Japan was 2.31 µg/g creatinine (16). Cadmium concentrations in urine of Itai-Itai disease patients measured in 1967 and 1975 were high, around 30 µg/g creatinine (17).

It is widely recognized that exposure to cadmium could cause kidney injury (18). The deleterious effects of cadmium on the reproductive system have been indicated, but the available evidence is not sufficient and equivocal. Previous studies including humans and animals have focused on the direct toxic effects of cadmium on reproductive organs. Cadmium-induced ovarian toxicity such as hemorrhagic necrosis and endothelial damage in the vessels has been reported in rats (19). In some animal studies, serum estradiol was measured as an indicator of ovarian damage in rats (20-22). A cadmium injection affected pregnancy estrogen in rats (21). Under in vitro cadmium exposure, testosterone, but not estradiol, was affected in proestrus rats (22). There is little epidemiologic information on the potential effects of cadmium exposure on the endocrine system in women. To our knowledge, this is the first report on the relationship between cadmium exposure and endogenous reproductive hormone levels among women.

We observed a moderate but significant positive association between urinary cadmium and serum testosterone levels. Cadmium exposure may alter the hormonal milieu. Although the P value for linear trend was statistically significant, there seems to be a threshold with testosterone. A similar tendency was noted for DHEAS. The reason for the increase in testosterone is unclear. The enhancement of the estrogenic status as a result of cadmium exposure may have influenced the mechanism that maintains the estrogen-androgen balance. Such an effect may appear only after a certain amount of exposure level. It is also possible that increased sex hormone–binding hormone may be associated with the increase in testosterone. Unfortunately, we did not measure gonadotropins and sex hormone–binding hormone.

There were no significant associations between urinary cadmium and serum estrone. Assessment of hormone status in postmenopausal women is subject to measurement errors. However, it seemed reasonable to assume that such measurement errors were not systematically related to urinary cadmium levels.

It has been hypothesized that exposure to an estrogenic compound may increase the risk of breast cancer (23). Only one study previously examined the association between cadmium exposure and breast cancer (24). In this study, the mean cadmium concentration in breast tissue in breast cancer patients did not differ significantly from that in the healthy controls. However, the sample size was small (n = 75). The authors stated that their results neither proved nor disproved the role of cadmium exposure in the development of breast cancer. In the present study, the observed modest association between urinary cadmium and testosterone can hardly be regarded as impairment of the reproductive endocrine function. However, the increased testosterone level associated with cadmium exposure may have implications for breast cancer risk. A higher testosterone level has been associated with increased breast cancer risk. A recent meta-analysis of nine prospective studies among postmenopausal women revealed about 1.4-fold risk of breast cancer associated with the doubling of testosterone level (8). According to their results, the estimated risk increase of breast cancer based on the mean testosterone levels shown in Table 3 is about 15% in women in the highest and the second highest categories of urinary cadmium as compared with those in the lowest category. This risk increase is not great but the number of cases that might be caused, assuming a causal relationship, may be relatively large because of the high proportion of these exposed groups. Urinary cadmium levels among the study subjects were much lower than those reported in men occupationally exposed to cadmium (7) or residents of cadmium-polluted areas (17). However, the concentration of cadmium in the environment has been increasing considerably and seems likely to continue to increase in the future (1).

Dietary data including alcohol intake were used for adjustment. As mentioned in the Materials and Methods, 21 women who had not completed the dietary data were excluded from the analysis. However, when we included these women into the analysis, the results were not altered substantially; the increase in testosterone in the highest as compared with the lowest category of urinary cadmium was 29% after controlling for age and BMI.

Due to the small sample size, the limited range of cadmium, and possible confounding with other unmeasured factors, the results should be considered preliminary. The cross-sectional nature of the present study precluded assessing the relationship of the timing of cadmium exposure to hormonal change. However, in postmenopausal women, circulating testosterone is much greater than estradiol; thus, testosterone is thought to have been involved in the etiology of depression, osteoporosis,
diabetes, and coronary artery disease in women (25). The association between testosterone and cadmium is interesting in light of the possible implication of testosterone into both breast cancer and these parameters of women’s health.

References
