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Associations of urinary cadmium with circulating sex hormone levels in pre- and postmenopausal Japanese women

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ABSTRACT

Background: Exposure to cadmium has been suspected as a risk factor for breast cancer. The present study examined the associations between urinary cadmium levels and circulating sex hormone levels that are linked to breast cancer risk in healthy women.

Methods: The study subjects were 396 premenopausal Japanese women who had regular menstrual cycles less than 40 days long and 207 postmenopausal Japanese women. Urinary cadmium was measured using spot urine samples. Plasma estradiol, testosterone, and dehydroepiandrosterone sulfate were measured. Additionally, the follicle-stimulating hormone, luteinizing hormone, and sex hormone-binding globulin were measured for premenopausal women.

Results: In premenopausal women, the urinary cadmium level either expressed in μg per liter or per g of urine creatinine was significantly inversely associated with total and free testosterone levels after controlling for age, body mass index, smoking status, alcohol intake, and the phase of the menstrual cycle. Total and free testosterone levels were 14.6% and 15.0% lower, respectively, in women in the highest quartile of urinary cadmium per g creatinine in those in the lowest quartile. In postmenopausal women, the urinary cadmium in μg per liter as well as per g creatinine was significantly inversely associated with the estradiol level after controlling for covariates. The estradiol level was 25.8% lower in women in the highest tertile of urinary cadmium per g creatinine than in those in the lowest tertile.

Conclusions: The data suggest inverse associations between urinary cadmium and the plasma estradiol or testosterone level in Japanese women.

1. Introduction

Exposure to cadmium has been suspected as a risk factor for breast cancer. Several epidemiological studies have shown that a high urinary cadmium level was associated with an increased risk of breast cancer (McElroy et al., 2006; Gallagher et al.,2010; Nagata et al., 2013; Strumylaite et al., 2014; Wei et al., 2015). A recent meta-analysis of eight epidemiological studies reported a 1.66-fold risk increase (95% confidence interval 1.50, 3.34) for each 0.5 μg/g creatinine increase in the cadmium level, although large prospective studies are still needed for confirmation (Larsson et al., 2015). Cadmium is a widespread environmental pollutant. The main sources of non-occupational exposure to cadmium in the general population include smoking, air, and food and water contaminated by cadmium (Järup et al., 1998). Cadmium has been classified as a human carcinogen (International Agency for Research on Cancer, 1993). The proposed mechanisms of cadmium carcinogen include induction of oxidative stress, apoptosis, and inhibition of DNA damage repair (Liu et al., 2009; Huff et al., 2007). In addition, cadmium exerts estrogenic activities since cadmium competes estradiol for binding to estrogen receptors and activates the receptors, leading to changes in gene expressions (Stoica et al., 2000). Cadmium acts like an estrogen in the uterus and mammary gland in rats (Johnson et al., 2003). However, we have to keep in mind that estrogenic effects differed dependent on animal species, and dose and route of administration (injection or oral treatment) of cadmium exposure (Höfer et al., 2009, Ali et al., 2010). There is evidence that higher estrogen or androgen levels are associated

Abbreviations: DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; LC-MS/MS, liquid chromatography-electrospray ionization tandem mass spectrometry; LH, luteinizing hormone; SHBG, sex hormone-binding globulin

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with an increased risk of breast cancer (The Endogenous Hormones and Breast Cancer Collaborative Group, 2002; Hankinson and Eliassed, 2010). Therefore, exposure to cadmium may increase sex hormone levels, which lead to the increased risk of breast cancer. Thus the relationship between cadmium exposure and endogenous sex hormone levels in women is of interest. Previously we were the first to pay attention to this relationship and found that a high urinary cadmium level was moderately but significantly associated with an increased testosterone level among postmenopausal Japanese women (Nagata et al., 2005). However, in that previous study, measurements using routine radioimmunoassay techniques failed to detect estradiol among postmenopausal Japanese women. Since then, only three studies have assessed the associations between blood or urinary cadmium level and circulating sex hormone levels in women (one study in premenopausal women Jackson et al., 2011) and two studies among postmenopausal women (Ali et al., 2014; Chen et al., 2015), and the measured sex hormones differed among studies.

In the present study, we assessed the association between urinary cadmium level and circulating sex hormone levels in both pre- and postmenopausal women. For postmenopausal women, we were able to measure their estrogen levels with a sensitive and reliable method using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-MS/MS).

2. Materials and methods

This study was carried out as a part of study designed to assess the relationships among lifestyle, environmental factors, and women's health, as described previously (Nagata et al., 2009). Study subjects were participants in a medical health check-up program provided by a general hospital in Gifu, Japan, between October 2003 and March 2006, including 1545 premenopausal and postmenopausal women (the response proportion: 74.5%). When the response proportion was calculated only for new visitors to the program during the study period, it was 83.2% (1103 out of 1325 individuals) (Nagata et al., 2009). The study was approved by the ethical board of the Gifu University Graduate School of Medicine and carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained from all individual participants included in the study.

The study subjects in this study were restricted to premenopausal women who were not pregnant or breastfeeding and had regular menstrual cycles of less than 40 days and postmenopausal women who had no menstrual cycle in the past 12 months. The feasible women were 501 premenopausal and 266 postmenopausal women. Women were excluded if they had cancer, diabetes mellitus, chronic hepatitis, or thyroid disease (22 premenopausal and 28 postmenopausal women), if they were using oral contraceptives, hormone therapy, or steroid (16 premenopausal and 8 postmenopausal women), or if their blood or urine samples were not obtained (7 premenopausal and 13 postmenopausal women). Two postmenopausal woman was further excluded because their estradiol levels suggested unreported estrogen use (>100 pg/mL). Twenty premenopausal women were excluded if her date of blood donation differed by more than 40 days from the onset of the last menses ($n=20$). Furthermore, 9 women (3 premenopausal and 8 postmenopausal women) with estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 were excluded. As a marker of renal function, eGFR was calculated by using the Modification of Diet in Renal Disease Study equation modified for Japanese (Matsuo et al., 2009) as follows: eGFR (mL/min/1.73 m^2) $=0.739\times 194\times$ (serum creatinine)$^{-1.094}\times$ age$^{-0.287}$. The final sample thus consisted of 396 premenopausal and 207 postmenopausal women.

Information regarding demographic characteristics, smoking status, menstrual and menopausal status, medical and reproductive histories, diet, and exercise was collected with a self-administered questionnaire during the health check-up. Alcohol and nutrient intakes were estimated using a validated food frequency questionnaire (Shimizu et al., 1999). Fasting blood and spot urine samples were obtained from participants at around 8:00 a.m. of the same day and plasma and urine was stored at -80 °C until the assay. Creatinine-adjusted cadmium level in overnight spot and 24-hour samples were much correlated (Akerstrom et al., 2012) and high reproducibility of measurements of urinary cadmium in spot samples during a 3-year period was reported (Arisawa et al., 1997).

Urinary cadmium was determined by flameless atomic absorption spectrometry (Z-5700 model, Hitachi, Tokyo) (Lagesson and Andrasko, 1979). The interassay coefficient of variation was less than 13.0%. Nine premenopausal (2.3%) and 2 postmenopausal (1.0%) women had urinary cadmium below sensitivity levels ($=0.4$ μg/L); thus, they were assigned with levels at half of assay sensitivity. In premenopausal women, plasma estradiol and testosterone were measured using electro chemiluminescent immunoassay with kits purchased from Roche Diagnostic Japan, Tokyo, Japan. Plasma dehydroepiandrosterone sulfate (DHEAS) was measured by chemiluminescent enzyme immunoassay using kits purchased from Beckman Coulter, Tokyo, Japan. Plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured using chemiluminescent immunoassay with kits purchased from Abbot Japan Co. Ltd., Tokyo, Japan. Sex hormone-binding globulin (SHBG) was measured using immunoradiometric assay with kits purchased from Diagnostic Products Corporation, Los Angeles, USA. The interassay coefficients of variation were $\leq 3.5\%$ for estradiol, $\leq 3.6\%$ for testosterone, $\leq 10.6\%$ for DHEAS, $\leq 7.9\%$ for SHBG, $\leq 5.7\%$ for LH, and $\leq 5.3\%$ for FSH. Free estradiol and testosterone were calculated using the measured estradiol, testosterone, albumin, and SHBG concentrations (Sodergard et al., 1983). In postmenopausal women, plasma estradiol and testosterone were measured by LC-MS/MS method (Yamashita et al., 2007) using reagents purchased from NIHS, Tokyo, Japan (estradiol) and Sigma-Aldrich Japan K.K (testosterone). DHEAS was measured by radioimmunoassay using kits purchased from Diagnostic Product Corporation, Tokyo Japan. The interassay coefficients of variation were $<7.6\%$ for estradiol, $<12.9\%$ for testosterone, and $<15\%$ for DHEAS. The sensitivities were 0.5 pg/mL for estradiol, 1.0 pg/mL for testosterone, and 1 μg/dl for DHEAS. Because of insufficient volume, plasma SHBG was not measured in one premenopausal woman and LH and FSH were not measured in 3 premenopausal women. DHEAS was not measured in one postmenopausal woman.

For statistical analysis, urinary cadmium levels expressed as μg per liter and per g of urine creatinine were transformed into logarithmical values. The relationships between urinary cadmium and plasma hormone levels were assessed by linear regression models using continuous variables. P-values for the linear trend were calculated in these models. Urinary cadmium was also examined as a categorical variable. The premenopausal women were divided into four equal groups according to quartile of urinary cadmium levels. Since the number of postmenopausal women was small, those subjects were divided into three equal groups according to tertile of urinary cadmium levels. The geometric means of hormones for each category were provided using analysis of covariance models. Age, body mass index, smoking status, and alcohol intake were included in all models as covariates. Urinary creatinine level was also included as a covariate for the models for urinary cadmium expressed in μg per liter. For premenopausal women, the phase of the menstrual cycle at blood donation was further included as a covariate. We estimated the date of the start