Short Communication

Urinary 6-Sulfatoxymelatonin and Mammographic Density in Japanese Women

Chisato Nagata,[1] Tomoko Matsubara,[6] Takeshi Hara,[2] Hiroshi Fujita,[2] Yasuko Nagao,[3] Satoru Yamamoto,[3] Chiken Shibuya,[3] Yoshitomo Kashiki,[3,4] and Hiroyuki Shimizu[1,5]

[1]Department of Epidemiology and Preventive Medicine and [2]Intelligence Image Information, Gifu University Graduate School of Medicine; [3]Gihoku General Hospital; [4]Asahi University School of Dentistry; [5]Sakihai Institute, Gifu, Japan and [6]Department of Information Culture, Nagoya Bunri University, Aichi, Japan

Abstract

A protective role of melatonin in the etiology of breast cancer has been suggested. The down-regulation of estrogen secretion by melatonin is thought to be a main cause of the link between melatonin and breast cancer risk. The present cross-sectional study examined whether the urinary 6-sulfatoxymelatonin (aMT6-s) level is inversely associated with mammographic density, which is regarded as a marker of breast cancer risk. The study subjects were 289 Japanese women (175 premenopausal and 123 postmenopausal women) who were recruited from participants in a mammographic breast cancer screening. The size of the total breast area and that of the dense area were measured quantitatively using an automated mammographic mass detection method. The concentration of aMT6-s was measured using first-void morning urine. In premenopausal women, the urinary aMT6-s level was significantly positively associated with percent density after controlling for covariates (P for trend = 0.02). There was no significant association between urinary aMT6-s level and the percent density in postmenopausal women. We found no evidence that the melatonin level is inversely associated with mammographic density. (Cancer Epidemiol Biomarkers Prev 2007;16(11):2509–12)

Introduction

One hypothesis claims that melatonin exerts protective effects against breast cancer. There is a general agreement that melatonin *in vivo* prevents the promotion and growth of spontaneous or chemically induced mammary tumors in rodents, whereas *in vitro* melatonin inhibits breast cancer cell proliferation and invasiveness (1). Evidence in humans is less direct. Thus far, two studies have prospectively assessed the association between melatonin and the risk of breast cancer in a direct manner (2, 3). Both studies used 6-sulfatoxymelatonin (aMT6-s), the main metabolite of melatonin in urine, which is a validated marker of the circulating melatonin level (4). A significant inverse association between the concentration of urinary aMT6-s and the risk of breast cancer was shown in one study (3), whereas the other did not confirm it (2). Night-shift work has been associated with an increased risk of breast cancer in several studies, including prospective studies (5), suggesting the implication of melatonin in this association. Exposure to light at night may increase the risk of breast cancer by suppressing the nocturnal production of melatonin by the pineal gland, which in turn could increase the release of estrogen by the ovaries (6).

Epidemiologic data consistently suggest that the percentage of mammographic dense area, which is referred to as mammographic density, is a marker of breast cancer risk (7). A high percent density has been associated with about a 3- to 6-fold increased risk of breast cancer. Mammographic density might then be expected to be inversely related to the melatonin level. In the present study, we examined the cross-sectional association between mammographic density and the concentration of urinary aMT6-s in Japanese women. To our knowledge, this relationship has not been evaluated in any other study.

Materials and Methods

Study subjects were recruited from participants in a breast cancer screening at a general hospital in Gifu, Japan, between June and December 2000. A total of 410 women who were free of breast cancer agreed to participate in the present study (response rate was 65.0%). This is a component study in a study of mammographic breast density (8). The details of the study have been described elsewhere (8). Informed consent was obtained from each woman. The study was approved by the institutional review board. A subset of this population was selected for a comparative study of mammographic

density among Caucasian and Japanese women in Hawaii and Japan (9).

Mammograms taken from either a mediolateral oblique (MLO) or craniocaudal (CC) view were obtained from each woman. Although the usual practice in Japan at the time of study period was to take MLO views only, CC views were available for 132 (44.3%) subjects for the use of the above-mentioned comparative study. All the mammograms were taken using the mammography machine Senographe DMR and read and recorded using the image reader Fuji Computed Radiography 3CS (model CR-IR331) and the recorder CR-LP415. Two of the present authors (H.F. and T.M.) developed an automated method for quantifying the amount and distribution of fibroglandular breast tissue density (10). The details of the procedure followed for the measurements have been described elsewhere (11). The percent density was calculated as the number of pixels within the dense area divided by the number of pixels for the entire breast area. The mean percentage of the density of both breasts was calculated for each woman. A total of 45 women from whom we obtained CC view films returned to the screening ~1 year later and provided the MLO view films. Using these films, we compared the mammographic measures between the two different views. The rank correlation between the CC and MLO views was 0.83 for the percent density. We also sent 131 MLO and 195 CC view mammograms to the Cancer Research Center of Hawaii to compare our mammogram measurements with those assessed by Maskarinec et al. (9) adopting the standard methods. Measurements were done independently. The rank correlation coefficients between their method and our methods were 0.80 (MLO view) and 0.80 (CC view) for the percent density.

The women responded to a self-administered questionnaire asking basic demographic characteristics, smoking and drinking habits, physical activity, and medical and reproductive history. A nurse epidemiologist visited participants and collected first-void morning urines on the next morning. The urine samples were frozen and stored at −80°C until assayed. Urinary aMT6-s was measured radioimmunologically using kits purchased from the IBL Laboratories. The intra-assay and interassay coefficients of variation were 9.8% and 15.3%, respectively. The urinary aMT6-s levels were expressed as urine aMT6-s/urine creatinine.

We excluded women who reported having cancer (*n* = 6) and women using hormone replacement therapy (*n* = 7) or contraceptive pills (*n* = 1) from the study. As the use of diuretics and β-blockers can affect urinary aMT6-s levels (12), we further excluded 61 women with the diagnosis of hypertension. The urine samples from 36 women were insufficient for the measurement. Body mass index (BMI) was missing for one woman. Hence, the remaining 298 women were the focus of this report. Women who had been without a menstrual cycle in the past 12 months or who were ages ≥55 years and did not report their menstrual status were classified as postmenopausal. The remaining women were classified as premenopausal.

Because the distribution of estimated percent density was positively skewed, a square root transformation was used in our study like other studies (e.g., refs. 13, 14). Analysis of covariance was used to estimate adjusted means of square root–transformed percent density

according to the quartile of urinary aMT6-s level and to test the linear trend. The mean values were squared and presented. Age, BMI, smoking status, mammogram view (MLO/CC), and day length (the number of hours of daylight between dawn and dusk) on the day previous to the urine collection were integrated as covariates into models. Furthermore, potential breast cancer risk factors, such as age at menarche, parity, age at first birth, age at menopause, alcohol intake, history of lactation, and family history of breast cancer among the first-degree relatives, were examined by including them into models as covariates. We also examined the association between urinary aMT6-s and the percent density as a categorized variable using analysis of covariance. Urinary aMT6-s level was transformed into logarithmic values. All statistical analyses were done using Statistical Analysis System (SAS Institute).

Results

Table 1 shows the characteristics of 298 women. Table 2 presents the means of the percent density according to the quartile of the urinary aMT6-s level. In premenopausal women, there was a significant association between the percent density and urinary aMT6-s level after controlling for age, BMI, smoking status, and day length on the day previous to the urine collection. The trend was significantly positive (*P* = 0.02). Additional adjustment for age at menarche, parity, age at first birth, alcohol intake, history of lactation, family history of breast cancer among first-degree relatives, and the day of the menstrual cycle did not alter the results substantially (*P* for trend = 0.04). In postmenopausal women, there was no significant association between the percent density and urinary aMT6-s level.

BMI was strongly associated with the percent density and moderately associated with urinary aMT6-s in premenopausal women (*r* = −0.47, *P* < 0.0001 and *r* = −0.10, *P* = 0.18, respectively) and postmenopausal women (*r* = −0.43, *P* = 0.0001 and *r* = −0.14, *P* = 0.12, respectively). We also repeated analysis stratified by BMI (≤23 and >23). The association between the percent density and urinary aMT6-s did not differ by level of BMI; for

Table 1. Characteristics of study subjects according to menopausal status

Variables	Premenopausal (*n* = 175)	Postmenopausal (*n* = 123)
Age (y)	43.1 (5.3)	55.8 (7.2)
BMI (kg/m^2)	22.7 (3.0)	23.2 (2.9)
Age at menarche (y)	12.9 (1.3)	14.0 (1.9)
Age at first birth (y)	25.3 (3.1)	24.4 (2.5)
Age at menopause (y)	—	47.5 (5.3)
Number of parity	2.3 (0.8)	2.3 (0.9)
Alcohol intake (mL/d)	6.8 (16.2)	2.7 (5.2)
Current smokers (%)	8.7	5.8
Ex-smokers (%)	2.3	0.0
Breast-feeding (%)	93.1	97.0
Family history of breast cancer among first-degree relatives (%)	5.1	1.6
Percent density (%)	35.3 (23.7)	20.5 (16.7)
aMT6-s (ng/mg creatinine)	43.2 (22.9)	40.0 (25.6)

NOTE: Values are means (SD) or percentages.

Table 2. Adjusted means with 95% confidence interval of percent breast density according to the quartile of urinary aMT6-s level

	Premenopausal				Postmenopausal			
	Range*	n	Mean[†] (95% CI)	Mean[‡] (95% CI)	Range*	n	Mean[†] (95% CI)	Mean[‡] (95% CI)
Q1 (low)	<25.6	44	21.1 (15.6-27.5)	22.7 (17.6-28.4)	<20.2	30	13.2 (8.5-19.0)	15.5 (10.7-21.3)
Q2	25.6-39.1	44	33.9 (27.1-41.6)	34.7 (28.5-41.6)	20.2-36.0	31	17.6 (12.2-24.1)	16.7 (11.7-22.6)
Q3	39.2-54.2	44	33.1 (26.3-40.6)	30.9 (25.0-37.3)	36.1-50.6	31	20.9 (15.0-27.7)	20.5 (15.1-26.7)
Q4 (high)	>54.2	43	36.2 (28.8-44.4)	35.7 (29.2-42.9)	>50	31	14.7 (9.6-20.9)	13.7 (9.1-19.1)
P for trend			0.008	0.02			0.58	0.86

Abbreviation: 95% CI, 95% confidence interval.
*Range of aMT6-s (ng/mg creatinine).
†Adjusted for age and mammogram view (MLO/CC).
‡Adjusted for age and mammogram view (MLO/CC), BMI, smoking status, and day length on the day previous to the urine collection.

example, in premenopausal women with low BMI, the mean estimates of the percent density were 32.2% and 44.7% for the lowest and highest quartiles of urinary aMT6-s, respectively (P for trend = 0.19), after controlling for the covariates. The corresponding figures for premenopausal women with high BMI were 13.7% and 22.2%, respectively (P for trend = 0.19).

When the percent density was examined as a categorized variable (<10%, 10-24%, 25-49%, and ≥50% for premenopausal women and <5%, 5-14%, 15-29%, and ≥30% for postmenopausal women), the results were essentially unaltered; the means of urinary aMT6-s levels were 30.4, 33.1, 39.7, and 39.5 ng/mg creatinine for the lowest to highest categories of the percent density, respectively (P for trend = 0.048), in premenopausal women. The corresponding figures in postmenopausal women were 30.1, 35.6, 34.7, and 31.2 ng/mg creatinine, respectively (P for trend = 0.89).

Discussion

Unexpectedly, we did not observe an inverse association between the percent density and urinary aMT6-s level. Endogenous melatonin did not seem to decrease mammographic density. We do not deny the potential protective effect of melatonin on breast cancer. However, our results suggest that, even if such an effect exists, it is not related with mammographic density. Mammographic density does not seem to be strongly associated with estrogen status. Most previous studies have observed no significant association between the percent density and serum estradiol level (e.g., refs. 15, 16). Bone density, which is a marker of cumulative exposure to estrogen, has been found to be unrelated to the percent density (17, 18). The only note exception involved increased density with hormone replacement therapy use, but the increase was small (19). In randomized clinical trials, the combined administration of estrogen and progesterone increased the percent density, but administration of estrogen alone did not (20). Boyd et al. (14) reported that the BMI was significantly associated with a risk of breast cancer after controlling for the percent density. They indicated that the estrogen-related factors associated with body size do not influence breast cancer risk through mammographic density. Considering the findings presented above, the authors emphasized the importance of considering both estrogen-related and nonestrogen-related factors when either is examined in

relation to risk of breast cancer (14). The down-regulation of estrogen secretion that controls the development of breast cancer is thought to be a potential mechanism of the role of melatonin as an anticancer agent (21). Thus, melatonin can be regarded as an estrogen-related factor. Some studies have examined the association between percent density and isoflavone or enterolactone, which is thought to have an influence on the estrogen metabolism and, thus, protective effects against breast cancer. These studies have also found no or only a slight positive association (22, 23). The results can also be explained by the absence of effects of estrogen-related factors on mammographic density.

Laboratory studies have shown that melatonin could act directly on breast cancer cells as a naturally occurring antiestrogen. In rodents, melatonin treatment decreases the number of epithelial structures of terminal, lateral, and alveolar buds, which are highly sensitive to tumor initiation by chemical carcinogens, and in contrast, it increases the number of epithelial structures representing the final stage of ductal growth, which are the most resistant to cancer initiation by exposure to carcinogens (21). Such a promotion of differentiation by melatonin may explain the increase of density in premenopausal women in this study.

The limitations of the study should be considered. The number of subjects, especially in postmenopausal women, was too small to allow consideration of all the confounders and leave sufficient power to detect any melatonin effect. In addition, subjects may have been homogeneous about urinary melatonin concentrations because they were recruited among the participants in a breast cancer screening. The percent density was strongly inversely correlated with BMI. The urinary aMT6-s level was also inversely related to BMI in our study, as it was in some other studies about the urinary melatonin level (12, 24). Exogenous melatonin can increase carbohydrate metabolism and decrease plasma leptin and ghrelin levels in animals (reviewed in ref. 25). Melatonin may act on energy balance and body weight regulation. Although our results from additional adjustment for BMI together with other covariates did not indicate a substantial confounding effect due to BMI, we cannot rule out the residual confounding effects of body size.

In summary, we found no evidence that the urinary melatonin level is inversely associated with mammographic density. The role of mammographic density as a surrogate marker of breast cancer should be further

evaluated. Studies are also needed to establish the association between the risk of breast cancer and melatonin as well as other potential estrogen-related factors.

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