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Sleep duration, spot urinary 6-sulfatoxymelatonin levels and risk of breast cancer among Chinese women in Singapore

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Abstract

We previously reported an inverse association between sleep duration and breast cancer risk in the prospective, population-based Singapore Chinese Health Study (SCHS) cohort (Wu *et al., Carcinogenesis* 2008;29:1244–8). Sleep duration was significantly positively associated with 6-sulfatoxymelatonin (aMT6s) levels determined in a spot urine, but aMT6s levels in breast cancer cases were lacking (Wu *et al., Carcinogenesis* 2008;29:1244–8). We updated the sleep duration–breast cancer association with 14 years of follow-up of 34,028 women in the SCHS. In a nested case–control study conducted within the SCHS, randomly timed, prediagnostic urinary aMT6s concentrations were compared between 248 incident breast cancer and 743 individually matched cohort controls. Three female controls were individually matched to each case on age at baseline interview (within 3 years), dialect group, menopausal status, date of baseline interview (within 2 years), date of urine sample collection (within 6 months) and timing of urine collection during the day (within 1 hr). Cox proportional hazards and conditional regression models with appropriate adjustment for confounders were used to examine the sleep– and aMT6s–breast cancer relationships. Breast cancer risk was not significantly associated with sleep duration; adjusted odds ratio (OR) for 9+ *vs.* ≤6 hr is 0.89 [95% confidence interval (95% CI) 5 0.64–1.22]. Prediagnostic aMT6s levels did not differ between breast cancer cases and matched controls; adjusted OR for highest *versus* lowest quartiles is 1.00 (95% CI 5 0.64-1.54). We conclude that sleep duration is not significantly associated with breast cancer risk reduction. Melatonin levels derived from randomly timed spot urine are unrelated to breast cancer. Randomly timed, spot urine-derived melatonin levels are noninformative as surrogates of nocturnal melatonin production.

Keywords

sleep duration; spot urinary melatonin; breast cancer; prospective; Singaporean Chinese

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Circadian disruption, as measured by sleep deficit and increasing exposure to artificial light at night and shift work, has been implicated as a breast cancer risk factor.[1] To date, results on prediagnostic urinary melatonin levels and breast cancer risk are based on levels of 6-sulfatoxymelatonin (aMT6s) collected in 24-hr,[2] 12-hr[3,4] or first-morning void specimens[5,6] from prospective studies conducted in the west. Supportive evidence has been reported from the Nurses' Health Study (NHS)[4,5] and postmenopausal women in the Italian Hormones and Diet in the Etiology of Breast Cancer Risk (ORDET) cohort[3] but not in premenopausal Italian women[4] or women in the Guernsey III cohort.[2] The relationship between sleep duration and breast cancer risk has been investigated in prospective studies that were conducted in the United States,[7] Finland[8] and Japan.[9] Results from two of these studies[8,9] showed a significant inverse association between sleep duration and breast cancer risk.

In 2008, we found that self-reported usual sleep duration determined at baseline was significantly inversely associated with subsequent risk of breast cancer in the prospective, population-based cohort of the Singapore Chinese Health Study (SCHS); this finding was observed primarily in lean (below median ≤ 23.3 kg/m^2) postmenopausal women.[10] In addition, urinary aMT6s levels, determined in a single-void spot urine (most were not first-morning voids), were statistically significantly higher (42%) among controls with 9+ hr versus those with <6 hr of sleep.[10] Similar to the sleep–breast cancer association, the positive association between aMT6s and hours of sleep was stronger and statistically significant in lean postmenopausal women. Our finding of a significant positive association between sleep duration and urinary aMT6 levels was unexpected because spot urine specimens are thought to be uninformative. However, it should be noted that although urinary aMT6s determined in first-morning voids correlated significantly with peak melatonin levels and with 24-hr serum melatonin levels[11] and the melatonin content of night urine specimens (collected between 2400 and 0700 hr) was two to three times higher than those collected during the day,[12] melatonin levels in urine samples collected during the day (*i.e.*, between 7 am and 7 pm) were not negligible but represented about 35% of the total melatonin content of 24-hr urine specimen collections.[12] Thus, although spot urine samples may not be ideal for estimating nocturnal melatonin production, prospectively conducted epidemiological studies using spot urine samples were lacking. Because of the compelling circumstantial evidence from our study showing a significant positive association between sleep duration and melatonin levels determined from randomly time spot urines[10] and the supportive data from Lang *et al.*,[12] we decided to further explore whether spot urines may provide meaningful information on the in vivo melatonin profile in humans in a well-established prospective cohort study, Thus, we conducted a follow-up study in the SHCS to address two questions. One, what is the relationship between sleep duration and breast cancer risk with a longer period of follow-up of the cohort? Confirmation of this finding with increased follow-up will enhance the credibility of the observation because sleep duration may change close to the time of cancer diagnosis. Two, does prediagnostic melatonin levels determined in randomly timed spot urine samples differ between women diagnosed with incident breast cancer (n = 248) versus their individually matched cohort controls (n = 743)?

Material and Methods

The study design and subject recruitment of the SCHS have been described.[13] Briefly, 35,303 Chinese women and 27,954 Chinese men aged 45–74 years belonging to the Hokkien or Cantonese dialect groups were enrolled between April 1993 and December 1998. As of 31 December 2010, <1% (n = 47) of cohort members were lost to follow-up. At recruitment, = extensive in-person interviews on lifestyle factors including the average number of hours of sleep in a 24-hr period, usual diet and reproductive history (for women only) were obtained.

We identified incident breast cancer cases through the nationwide cancer registry[14] and used established breast cancer histological and tumor staging protocol.[15] As of December 31, 2009, 820 incident breast cancers have been identified among the 34,028 at-risk female participants [this excluded cases (n = 51) who were diagnosed <2 years of follow-up, as in the last analysis, and 1,275 of the 35,303 women who had prevalent cancer at the time of study enrollment]. Thus, the current analysis on sleep duration and risk is based on 769 breast cancer cases who were diagnosed 2 or more years after enrollment; an addition of 244 breast cancer cases and 184,299 person-years (PY) to our 2008 analysis of 525 breast cancer cases and 270,628 PY.[10] A total of 248 of these 769 women had prediagnostic urine specimens (see below).

A Biospecimen Subcohort was begun in April 1994 by asking a random 3% sample of enrollees to provide blood or buccal cells and spot urine samples. This recruitment effort was extended to all surviving cohort participants in January 2000.[16] At the completion of this effort in April 2005, biospecimens were collected from 32,535 cohort participants (~60% consent rate). The present biomarker study included ≤248 incident breast cancer cases and three individually matched cohort controls for each case (one case had only two matched controls). The matching criteria were age at (within 3 years) and date of baseline interview (within 2 years), gender, dialect group (Cantonese *vs.* Hokkien), menopausal status at urine collection, date of urine collection (within 6 months) and timing of urine collection during the day (within 1 hr). Concentrations of aMT6s, the main metabolite of melatonin in urine, were quantified using the same methods as in our previous study.[10]

Statistical analysis

We calculated PY from the baseline questionnaire to the date of breast cancer diagnosis, death or December 31, 2008, whichever was sooner. As in our previous study,[10] Cox proportional hazards regression methods were used to examine the association between categories of sleep duration (≤6, 7, 8 and 9+ hr/day) and breast cancer risk, measured by estimated relative risks (reported as odds ratios, ORs) and their corresponding 95% confidence intervals (95% CIs). Relevant demographic factors including age (years) at recruitment, year of recruitment (1993–1998), dialect group (Cantonese and Hokkien), education (no formal education, primary school, secondary school or higher) and established risk factors for breast cancer including age when period became regular (<12, 13–14, 15–16, 17+ years or periods never became regular), number of live births (none, 1–2, 3–4 and 5+), body mass index (BMI, continuous, kg/m^2) and menopausal status were adjusted for in the analysis. Results were similar with additional adjustment for factors that appeared to influence sleep duration [including tobacco smoking (never and ever) and alcohol intake (nondrinker, monthly, weekly and daily) and history of diabetes.[10] We show the results without these further adjustment (Table 1).

For the nested case–control study, the analysis of covariance method was used to examine case–control differences in urinary aMT6s and to compare urinary aMT6s by time of urine collection among controls. Logarithmically transformed aMT6s values were used because of the variable's skewed distribution. Urinary aMT6s was also analyzed as categorical variables, with the distribution in controls used to define the categories. Conditional logistic regression methods were used in computing risk estimates associated with varying categories of aMT6s. We also conducted subgroup analyses, stratified by BMI, time interval between diagnosis and urine collection. p values <5% are considered statistically significant, and all p values quoted are two sided.

Results

Table 1 shows that there was a nonsignificant reduction in breast cancer risk with increasing hours of sleep (p trend = 0.68). There was no evidence of risk reduction in premenopausal women (data not shown), and among postmenopausal women, those with 9+ hr of sleep showed a RR of 0.81 (0.55–1.18; p trend = 0.21) compared to those with ≤6 hr of sleep. Any reduction in risk with increasing duration of sleep among leaner (BMI ≤ 23.2 kg/m^2) postmenopausal women was not statistically significant (p trend 0.33).

Table 2 shows baseline characteristics of cases (n = 248) and their matched controls (n = 743) in the nested case–control study. Cases were more likely to be nulliparous (p = 0.009) and had higher BMI (p = 0.026) compared to controls. The mean time between urine collection and diagnosis was 3.3 years (±2.1; range: 0.5–147 months). Similar to our previous findings,[10] aMT6s concentrations decreased significantly with increasing interval between early morning (8 am) and time of urine collection (p trend < 0.0001; Table 3). However, in contrast to our previous findings,[10] duration of sleep was unrelated to melatonin levels in control women (p trend = 0.38). Furthermore, lean (below median BMI) postmenopausal women demonstrated no association between hours of sleep and aMT6s levels (p trend = 0.22), whereas heavy (above median BMI) postmenopausal women actually showed a marginally significant positive association between hours of sleep and aMT6s levels (p trend = 0.059; Table 3).

Age-adjusted aMT6s concentrations did not differ between breast cancer cases (1.26 ng/mg Cr) and controls (1.24 ng/ mg Cr; p = 0.77). Breast cancer risk was unrelated to quartiles of urinary aMT6s in all subjects (p trend 0.90) and in postmenopausal women (p trend = 0.96; Table 4). Given that melatonin may influence breast=cancer development via an estrogen-driven pathway, we investigated whether this association in postmenopausal women may be modified by BMI. We found no effect modification by BMI (stratified along the median, ≤23.2 kg/m^2; Table 4). The melatonin–breast cancer association did not differ by duration of followup (<4 *vs.* 4+ years) or by timing of urine donation during the day (before or after noon; data not shown).

Discussion

In 2007, the International Agency for Research on Cancer concluded that “shift work that involves circadian disruption is probably carcinogenic to humans.”[1] Melatonin has been proposed to be a key biological intermediary of chronodisruption.[17-19] Our study is the first one in an Asian population to investigate two surrogate measures of circadian disruption, sleep duration and prediagnostic melatonin levels from spot urine (most were not first-morning voids), and risk of breast cancer. Our results show that sleep duration is not significantly associated with breast cancer risk. The data also clearly show a lack of association between breast cancer risk and melatonin levels derived from randomly timed spot urine samples and conclusively refute our earlier results suggesting that this biomarker may be informative as a surrogate of nocturnal melatonin production.

Three prospective studies have investigated the sleep duration–breast cancer relationship.[7-9] No association was found in the NHS with more than 4,200 incident breast cancers, but among participants who reported the same sleep duration patterns in 1986 and 2000, there was a trend toward increasing risk with increasing sleep duration.[7] In contrast, a significant inverse association between risk and sleep duration was reported in two studies, each with fewer than 250 breast cancer cases.[8,9] Our sleep duration–breast cancer finding with nearly 14 years of follow-up data is qualitatively consistent with our previous findings,[10] but the risk estimates determined from the current, expanded data set are markedly weaker and none

of them achieved statistical significance. Methodological limitations of our study include (*i*) that information on sleep duration was obtained only at baseline and (*ii*) that our question on sleep pattern is crude, without details such as usual time to bed or rising, lighting conditions during sleep and other parameters.

The relationship between prediagnosis melatonin levels and breast cancer risk has been investigated in the NHS,[5,6] the ORDET cohort[3,4] and the Guernsey III cohort.[2] Supportive results were reported for both premenopausal and postmenopausal women from the NHS, which collected first-morning[5,6] samples, and for postmenopausal women in the ORDET cohort based on 12-hr urine specimens.[3] High melatonin levels were significantly positively related to breast cancer risk in premenopausal Italian women, but this finding appeared to be influenced by the timing between urine collection and diagnosis.[4] Breast cancer risk was unrelated to melatonin levels in the Guernsey III cohort, which collected 24 hr samples.[2] Although our study sample size in the melatonin analysis is comparable to previous studies conducted in western populations and the prevalence of potential confounders (*e.g.*, menopausal hormone use, smoking, alcohol and high BMI) is low in our study population, we differ from those published results in that our melatonin levels were derived from randomly timed spot urine specimens.

In this investigation and our previous study,[10] identical methods were used to measure melatonin levels and they were conducted by the same technician in Dr. Stanczyk's laboratory. In both studies, samples collected before 10 am showed significantly higher melatonin levels than samples collected after 12 noon; levels were 1.8 times higher in the current (Table 3) and 2.4 times higher in the previous study.[10] However, we found essentially no difference in urinary melatonin levels by hours of sleep in our study (Table 3), whereas in the previous study,[10] melatonin levels were significantly higher (42%) in women who slept 9+ hr compared to those who slept ≤6 hr/day. We repeated the sleep duration–melatonin analysis among the 198 control subjects who donated urine samples before 10 am as they were more likely to represent first-morning voids. We found that sleep duration was, in fact, inversely associated with urinary melatonin levels (aMT6s levels were 1.88, 1.85, 1.23 and 1.32 for ≤6, 7, 8 and 9+ hr of sleep, respectively, p trend 0.018) in this subgroup of women. Taken as a whole, our=current data in comparison to our earlier findings[10] lead us to conclude that melatonin measurements derived from randomly timed spot urine specimens are noninformative as surrogates of nocturnal melatonin production. Randomly timed, spot urine samples are not an appropriate medium for a surrogate marker of melatonin profile in humans.

Epidemiologic data on endogenous melatonin and breast cancer risk remain sparse because of the lack of prediagnostic urine specimens from prospective cohort studies. A recent methodologic study measured melatonin levels in serum, plasma and urine samples collected in healthy men.[20] A significant correlation between urine and serum melatonin levels ($r = 0.46$, $p = 0.008$) was found; the serum samples were derived from blood collected before noon, whereas urine samples were collected for the same participants during a 24-hr period after blood collection. Plasma and serum melatonin levels ($r = 0.97$, $p < 0.0001$) were also significantly correlated; about 70% of the paired samples were morning blood draws and the others were afternoon draws. These results suggest that serum/plasma melatonin measurements could be used in large epidemiologic studies. Given the potential importance of circadian rhythm hypothesis in the development of breast and other cancers, use of serological melatonin measurements in epidemiologic investigations of the melatonin-breast cancer hypothesis should be considered.

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What's new?

Sleep duration, spot urinary 6-sulfatoxymelatonin levels and risk of breast cancer among Chinese women in Singapore Anna H. Wu, Frank Z. Stanczyk, Renwei Wang, Woon-Puay Koh, Jian-Min Yuan and Mimi C. Yu Melatonin has been proposed as a key mediator of chronodisruption, a disturbance that may underlie the probable carcinogenic effects of shift-work in humans. In this prospective study, sleep duration and pre-diagnostic melatonin levels were investigated in association with breast cancer risk in an Asian population. Risk was not significantly related to sleep duration or melatonin levels derived from randomly timed spot urine sample suggesting that randomly timed, spot urine is not appropriate as a surrogate marker of melatonin profile in humans, despite earlier evidence supporting this possibility. Epidemiology

Table 1

Breast cancer risk (95% CI) by sleep duration in all women and postmenopausal women at baseline

	All subjects			Postmenopausal women at baseline		Postmenopausal women at baseline			
						BMI ≤ 23.2		BMI > 23.2	
Sleep hours	**CA**	**PY**	**OR[1] (95% CI)**	**CA**	**OR[1] (95% CI)**	**CA**	**OR[1] (95% CI)**	**CA**	**OR[1] (95% CI)**
≤6	257	152,739	1.00	193	1.00	80	1.00	113	1.00
7	259	151,597	1.00 (0.84-1.19)	162	0.91 (0.74-1.12)	67	0.89 (0.64-1.23)	95	0.95 (0.72-1.25)
8	208	120,504	1.00 (0.84-1.21)	129	0.90 (0.72-1.13)	50	0.85 (0.60-1.21)	79	1.03 (0.77-1.38)
9+	45	30,087	0.89 (0.64-1.22)	31	0.81 (0.55-1.18)	12	0.82 (0.45-1.51)	19	0.89 (0.55-1.45)
p trend			0.68		0.21		0.33		0.90

[1]Adjusted for age at recruitment, year of recruitment (1993-1998), dialect group (Cantonese and Hokkien), education (no formal education, primary school, secondary school or higher), age when period became regular, parity, BMI (continuous, kg/m^2) and menopausal status (only in analysis for all subjects).

Table 2

Nested case–control study: Age-adjusted baseline characteristics of breast cancer cases and control women

	Cases,*n* = 248	Control,*n*= 743	p value
Mean age (years)[1]	61.3 6±8.0	61.6 6±7.9	0.54
% Secondary school education	27.7	25.6	0.48
% Postmenopausal[1]	93.1	92.6	0.81
% Cantonese	56.0	56.1	0.98
%≤12 age at menarche	11.8	16.2	0.09
% Nulliparous	12.0	6.9	0.01
% First live birth, age ≤ 20 years	14.0	16.1	0.43
% Below median BMI (≤23.2 kg/m^2)	46.7	54.9	0.025
% Diabetic	9.0	6.6	0.20
% Ever smoked	5.8	6.1	0.85
% Weekly alcohol	2.4	4.2	0.21
% No weekly moderate, vigorous or strenuous physical activity	71.0	70.8	0.94
% Ever use menopausal hormones[1]	18.3	15.7	0.33
%≤6 hr *vs.*9+ hr of sleep	30.4*vs.*4.4	32.9*vs.*6.9	0.32

[1]At the time of urine specimen collection.

Table 3

Geometric means of urinary aMT6s (μg/g Cr) by time of specimen collection and by sleep duration in all control women and postmenopausal control women by BMI

	All control		
Time of specimen collection-control[1]	**No.**	**aMT6s Mean**	**95% CI**
8:00–8:59	1	6.00	0.93–38.56
9:00–9:59	197	1.62	1.42–1.85
10:00–10:59	187	1.28	1.12–1.47
11:00–11:59	191	1.14	1.00–1.31
12:00–12:59	149	0.97	0.83–1.13
13:00–16:00	18	0.70	0.46–10.9
p trend			<0.0001

Hours of sleep control[2]	**All controls**			**Postmenopausal women BMI ≤ 23.2**			**Postmenopausal women, BMI ≤ 23.2**		
	No	**aMT6s Mean**	**95% CI**	**No**	**aMT6s Mean**	**95% CI**	**No**	**aMT6s Mean**	**95% CI**
≤6	245	1.15	1.02–1.30	118	1.21	1.03–1.44	115	1.13	0.94–1.36
7	242	1.30	1.14–1.46	131	1.26	1.08–1.48	95	1.36	1.12–1.67
8	205	1.28	1.13–1.46	100	1.03	0.86–1.24	84	1.51	1.22–1.88
9+	51	1.20	0.92–1.55	25	1.09	0.76–1.57	21	1.49	0.97–2.28
p trend			0.380			0.22			0.059

[1] Adjusted for age and case/control status (if applicable).

[2] Adjusted for age, case/control status (if applicable) and time of specimen collection.

Table 4

Breast cancer risk (95% CI) by urinary aMT6s in all subjects and in postmenopausal women only

	All subjects			Postmenopausal women only[1]			Postmenopausal women only[1]					
							BMI ≤ 23.2			BMI > 23.2		
Urinary aMT6s	Case	Control	OR[2] (95% CI)	Case	Control	OR[2] (95% CI)	Case	Control	OR[2] (95% CI)	Case	Control	OR[2] (95% CI)
First quartile	58	186	1.00	51	173	1.00	22	93	1.00	29	80	1.00
Second quartile	69	186	1.18 (0.78–1.78)	66	172	1.27 (0.83–1.95)	28	101	1.19 (0.63–2.24)	38	71	1.49 (0.83–2.67)
Third quartile	63	186	1.14 (0.75–1.74)	59	172	1.20 (0.77–1.87)	30	96	1.41 (0.75–2.67)	29	76	1.09 (0.59–2.00)
Fourth quartile	58	185	1.00 (0.64–1.54)	54	172	1.04 (0.65–1.64)	26	84	1.38 (0.71–2.69)	28	88	0.94 (0.51–1.75)
p trend			0.90			0.96			0.29			0.61

[1] Excluded 18 premenopausal cases and 54 premenopausal controls.

[2] From conditional logistic regression with adjustment for age at recruitment, date of recruitment (1993–1998), dialect group (Cantonese and Hokkien), education (no formal education, primary school, secondary school or higher), menopausal status (only in analysis in all subjects), number of live births (none, 1–2, 3–4 and 5+) and BMI (continuous, kg/m^2).