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Urinary melatonin and risk of ovarian cancer

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Abstract

Purpose—Melatonin has anti-carcinogenic properties, including modulation of estradiol production, cell-cycle regulation, and promotion of apoptosis. Urinary melatonin has been inversely associated with breast cancer in some studies, but the association with ovarian cancer has not been investigated.

Methods—We measured urinary 6-sulfatoxy-melatonin (aMT6s) in nested ovarian cancer case-control studies in the Nurses' Health Study (NHS; n=100 cases; 199 controls) and NHSII (n=52 cases; 105 controls); samples were mainly from first morning voids. Controls were matched to cases on year of birth, menopause status, use of menopausal hormone therapy, and urine collection characteristics. We evaluated the association of tertiles of aMT6s, corrected for creatinine concentrations, with risk of ovarian cancer using conditional logistic regression. Models were adjusted for key ovarian cancer risk factors, and we additionally evaluated adjustment for usual sleep duration, snoring, and history of rotating night shift work.

Results—aMT6s was not significantly associated with risk of ovarian cancer. In multivariable models, the odds ratio comparing the highest tertile of aMT6s to the lowest was 0.79; 95% confidence interval (CI): 0.40–1.56 in the NHS and 2.88; 95% CI: 0.97–8.52 in the NHSII. Additional adjustment for sleep habits and night shift work had little impact on the observed results. We observed no clear association between urinary melatonin and ovarian cancer risk.

Conclusions—These results are consistent with our previous study in which we reported no association between night shift work and ovarian cancer; however, given the small sample size in our study, additional evaluation in larger studies is warranted.

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The authors have no conflicts of interest to disclose.

Keywords

melatonin; circadian rhythm; ovarian cancer

Background

Although night shift work has been classified by the International Agency for Research on Cancer as a probable human carcinogen [1], the association of shift work with ovarian cancer is unclear [2–4]. In the Nurses' Health Study (NHS) and NHSII, there was no clear relationship between rotating shift work and risk of ovarian cancer [2], but a prospective study of fatal ovarian cancer and a case-control study observed a modest (12–24%) increased risk for ever (versus never) night shift work [3, 4], with stronger associations (27–28% increased risk) for rotating shift work. Inconsistencies may be due in part to differences in associations by age, as well as different methods of assessing shift work.

Shift work is thought to influence carcinogenesis through reduced melatonin production by the pineal gland due to light exposure at night [5, 6]. Thus, assessing the association between circulating melatonin levels (using a proxy measure in first morning urine) and cancer may provide the mechanistic underpinning for any effects of shift work on cancer risk. Indeed, generally strong associations between urinary 6-sulfatoxymelatonin levels and breast and prostate cancer risk have been observed in several studies (reviewed in [7]). Biologic evidence supports a role of melatonin in ovarian carcinogenesis. Some, but not all, studies have reported that melatonin (and its metabolites) can inhibit the growth of several ovarian cancer cell lines, although the effect may depend on the estrogen receptor (ER) α to ER β ratio and on experimental conditions [8–14]. Further, in turkey hens and rats, melatonin appeared to reduce ovarian tumor growth [15, 16]. Melatonin, which can be synthesized by the ovary and is found in follicular fluid, may also influence ovarian function in premenopausal women by reducing luteinizing hormone and increasing progesterone production [17, 18].

One study of 23 cases of endometrial, ovarian, or cervical cancer observed no difference in melatonin circadian rhythms compared to controls [19]; however, no studies have prospectively examined urinary melatonin excretion and risk of ovarian cancer. Therefore, we measured 6-sulfatoxymelatonin (aMT6s) in first morning or spot urine samples in the NHS and NHSII in a nested case-control study of ovarian cancer.

Methods

Study population

This analysis is based on data from a nested case-control study in the NHS and NHSII. The NHS cohort was established in 1976 among 121,700 U.S. female registered nurses, aged 30–55 years, and the NHSII was established in 1989 among 116,430 female registered nurses, aged 25–42 years. Women in both cohorts completed an initial questionnaire and have been followed biennially by questionnaire to update exposure status and disease diagnoses. In 2000–2002, 18,743 NHS participants (aged 53–80 years) provided a spot urine sample (93% were first morning samples) and completed a short questionnaire. Briefly, women arranged

to collect a urine sample and ship it with an icepack, via overnight courier, to our laboratory where it was aliquoted and stored in liquid nitrogen freezers. Among NHS participants who provided biospecimens, follow-up was 97% complete in 2012. Between 1996–1999, 29,611 NHSII participants (aged 32–54 years) provided a spot urine samples (80% were first morning samples) and completed a short questionnaire [20]. Briefly, premenopausal women (n=18,521) who had not taken hormones, been pregnant, or lactated within six months provided a urine sample collected 7–9 days before the anticipated start of their next cycle (luteal phase). Other women (n=11,090) provided an untimed sample. Samples were shipped and processed identically to the NHS samples. Among NHSII participants who provided biospecimens, follow-up was 88% complete in 2013.

Cases had no previous history of cancer, except non-melanoma skin cancer, before urine collection and were diagnosed with ovarian cancer after urine collection and before June 1, 2012 (NHS) and June 1, 2011 (NHSII). Overall, 152 cases of primary invasive epithelial ovarian or peritoneal cancer (100 in NHS and 52 in NHSII) were confirmed by medical record review among participants in the urine collection. Cases were matched to two controls (who had intact ovaries at the time of the case diagnosis) on menopause status at collection and diagnosis (premenopausal, postmenopausal, unknown), age (± 1 year), month of urine collection (± 1 month), and use of menopausal hormone therapy at urine collection (yes, no). For NHSII cases with timed samples, we additionally matched on day of the luteal urine sample collection (date of next menstrual cycle minus date of sample collection, ± 1 day).

Laboratory assays

Urinary aMT6s and creatinine were assayed at the General Clinical Research Center Core Laboratory at the Brigham and Women's Hospital via the Bühlmann 6-SMT ELISA system (ALPCO, Windham, NH), which is a competitive immunoassay using an antibody-capture technique with a lower detection limit of 0.8 ng/ml. aMT6s assays were creatinine-standardized to account for differences arising from variations in urine concentrations. Creatinine was measured using a modified Jaffe method. Case-control sets and samples from the same study were assayed together, ordered randomly, and labeled to mask case-control status and quality control status. Coefficients of variation (CV) for aMT6s were <8% and for creatinine were <7%.

Statistical Analysis

Statistical outliers (N=9) were excluded using the generalized extreme studentized deviate many-outlier detection procedure [21]. In the NHS/NHSII, relative risks (RR) and 95% confidence intervals (CI) were determined using conditional logistic regression comparing tertiles (cut points based on control distribution) of aMT6s concentrations adjusted for creatinine. Trend tests were assessed using tertile medians. Analyses were adjusted for oral contraceptive (OC) use (never, <5 yrs, 5+ yrs), tubal ligation (yes, no), parity (continuous), smoking status (never, former, current), family history of ovarian cancer (yes, no), and body mass index (BMI) at urine collection (continuous). In a separate model, we additionally adjusted for usual sleep duration (6, 7, 8 hrs/night), history of snoring (ever, never), and history of rotating shift work (yes, no). NHS and NHSII were analyzed separately due to the

lack of overlap in age distribution at sample collection; because the results were heterogeneous ($p\text{-het}=0.04$), we did not combine the results from the two cohorts. We also performed a sensitivity analysis limited to first morning urine samples.

Results

Overall, there were 100 cases of ovarian cancer and 199 controls with assay values in the NHS and 52 cases and 105 controls in NHS2. Cases were more likely to be nulliparous, use OCs for shorter durations, not have a tubal ligation and have a family history of ovarian cancer compared to controls (Table 1). Cases and controls had similar distributions of sleep duration; however cases were more likely to report ever snoring than controls. In the NHS, cases were more likely to report a history of rotating shift work than controls, but the opposite pattern was observed in NHSII.

In the NHS, in which 98% of the women were postmenopausal, we observed a suggestive decreased risk comparing the top versus bottom tertile in the multivariate analysis ($RR=0.79$, 95%CI=0.40–1.56; $p\text{-trend}=0.49$). Adjusting for sleep related factors and rotating shift work, slightly strengthened the RR to 0.76 (95%CI=0.37–1.55; $p\text{-trend}=0.44$). Conversely, in the NHSII, in which ~75% of women were premenopausal and 15% postmenopausal (~10% were of unknown status) at sample collection, we observed a suggestive increase in risk comparing women in the top versus bottom tertile (multivariate $RR=2.88$, 95%CI=0.97–8.52; $p\text{-trend}=0.04$). The multivariate association appeared stronger than in the model only accounting for matching factors; this increase was primarily due to adjustment for BMI and smoking. The association was modestly attenuated when adjusting for sleep related factors and shiftwork ($RR=2.66$, 95%CI=0.87–8.11; $p\text{-trend}=0.07$). Results were similar, although more unstable, when the analysis was limited to first-morning urine samples (Supplemental Table).

Discussion

Overall in this first prospective study of urinary aMT6s and risk of ovarian cancer, we did not observe a clear relationship. While there was a suggestive inverse association in the NHS (primarily postmenopausal women and >55 yrs), there was a suggestive positive association in the NHSII (primarily premenopausal women and <55 yrs), though the risk estimates were not statistically significant in either group. This suggests that melatonin may differentially influence risk by menopausal status or by age or possibly due to having fewer serous cases in NHSII (64% serous in NHS and 50% in NHSII). However, the sample sizes were small in both cohorts precluding additional analyses to address this apparent disparity, including restricting to only premenopausal women in NHSII, stratifying by postmenopausal hormone use status in NHS, or considering associations by histologic subtype.

In a recent meta-analysis of urinary aMT6s and breast cancer risk, there was a significant inverse association for postmenopausal breast cancer (RR , top versus bottom quartile=0.68), but no association for premenopausal cases (comparable $RR=1.05$), although this difference was not statistically significant ($p\text{-heterogeneity}=0.09$) [22]. Similarly, in analyses of NHS and NHSII, there was a statistically significant inverse association between urinary

melatonin and breast cancer risk (predominantly postmenopausal women), but no association among NHSII participants (predominantly premenopausal women) [23, 24]. This may suggest that melatonin has hormonal effects on carcinogenesis and that these effects may vary with age/menopausal status. Studies suggest that melatonin can suppress aromatase activity in breast tumors [25] and in ovarian tissue in animal models simulating perimenopause [26]. Melatonin may also be an ER modulator [5, 8]; this effect may depend on the ratio of ER α to ER β , with high ER β levels abrogating the ability of melatonin to modulate ER α [8, 27]. Interestingly, a study of ER β expression in serous ovarian tumors, reported that cytoplasmic ER β 2 expression was associated with worse survival; more than half of women 54 years old had cytoplasmic ER β 2 expression, but only a third of older women expressed this marker [28]. Additional research is needed to better elucidate the role of melatonin in regulating estrogen signaling pathways in the presence of ER subtypes.

The main strength of this study was the collection of primarily first morning urine samples and prospective follow-up for ovarian cancer. However, our results are limited by the small number of women diagnosed after urine collection, the very different age distribution at urine collection between the two cohorts, and the lack of information on use of melatonin supplements. This limited our ability to stratify by factors such as postmenopausal hormone use and to examine associations with specific histologic subtypes. The latter may be particularly important as DBMA-induced rats exposed to melatonin had 20% lower tumor mass versus controls and a reduced incidence of serous tumors specifically [15]. Further, in a case-control study of shiftwork, the association for ever versus never shiftwork was stronger for serous tumors compared to other subtypes [3]. Interestingly, in our study, a slightly higher proportion of NHS cases were of serous histology compared to NHSII. Additionally, specimens assayed in this study were collected up to 14 years prior to the cases' diagnosis (mean 4.9 years in NHS; 5.6 years in NHSII). It is possible that specimens collected closer in time to diagnosis would have yielded a stronger association. Similarly, it is possible that a one-time measure of urinary melatonin may not reflect long-term exposure to circulating melatonin, thus attenuating results. However, among 80 NHSII participants who donated urine once per year over 3 years, the intraclass correlation for creatinine-adjusted melatonin was 0.72 [29], suggesting that a one-time measure reasonably reflects longer-term melatonin levels and that the long delay between collection and diagnosis did not greatly attenuate the results.

Overall our results do not provide strong evidence of a role for melatonin in ovarian cancer risk, although the sample size was limited. However, given the suggestive inverse association in the NHS (primarily postmenopausal women) in our analyses and prior studies generally supporting an inverse association of melatonin with postmenopausal breast cancer risk, additional studies of ovarian cancer with larger sample sizes are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Characteristics of ovarian cancer cases and controls in NHS and NHSII at the time of urine collection.

	NHS		NHSII	
	Controls n=199	Cases n=100	Controls n=105	Cases n=52
Age, mean (SD) ^a	67.9 (6.4)	67.8 (6.3)	44.9 (4.7)	44.9 (4.7)
Log aMT6s, mean (SD)	-1.2 (0.8)	-1.2 (0.7)	-1.1 (0.7)	-1.0 (0.6)
Years between sample collection and diagnosis, mean (SD) ^a	N/A	4.9 (3.5)	N/A	5.6 (5.2)
Body mass index, mean (SD)	25.6 (4.2)	25.3 (4.3)	26.4 (6.1)	27.5 (7.6)
Duration of oral contraceptive use, %				
Never	51	48	13	14
<1 yrs	11	16	11	9
1-<5 yrs	21	21	41	49
5 yrs	17	14	34	28
Number of children, %				
Nulliparous	3	4	22	33
1 child	7	3	10	18
2 children	27	34	38	35
3 children	25	26	20	9
4+ children	39	32	10	6
Tubal ligation, %	22	19	31	11
Family history of ovarian cancer, %	3	6	2	0
Usual duration of sleep, %				
6 hrs/night	18	14	23	20
7 hrs/night	40	41	49	50
8 hrs/night	42	44	28	30
Ever snore, %	28	39	50	56
History of rotating night shift work, %	53	61	70	62
Menopause status/use of menopausal hormone therapy (HT), %				
Premenopausal	1	1	74	78
Postmenopausal/no HT use	38	30	7	7
Postmenopausal/HT user	59	68	8	8
Unknown	1	0	11	7
Smoking status, %				
Never	47	51	73	61
Former	48	46	19	32
Current	4	3	8	7

^aValue is not age adjusted

Table 2
Associations between urinary melatonin (aMT6s) and risk of ovarian cancer in the NHS and NHSII.

NHS									
Tertile of aMT6s ^d	Controls	Cases	Model 1 ^a		Model 2 ^b		Model 3 ^c		
			OR	95%CI	OR	95%CI	OR	95%CI	
1	67	36	1.00	(ref.)	1.00	(ref.)	1.00	(ref.)	
2	67	33	0.88	(0.48–1.59)	0.89	(0.47–1.66)	0.88	(0.46–1.67)	
3	65	31	0.83	(0.43–1.60)	0.79	(0.40–1.56)	0.76	(0.37–1.55)	
p-trend ^e			0.56		0.49		0.44		

NHSII									
Tertile of aMT6s ^d	Controls	Cases	Model 1 ^a		Model 2 ^b		Model 3 ^c		
			OR	95%CI	OR	95%CI	OR	95%CI	
1	34	10	1.00	(ref.)	1.00	(ref.)	1.00	(ref.)	
2	33	19	2.02	(0.85–4.82)	3.17	(1.04–9.62)	3.21	(1.07–9.61)	
3	38	23	1.97	(0.88–4.39)	2.88	(0.97–8.52)	2.66	(0.87–8.11)	
p-trend ^e			0.09		0.04		0.07		

^a Conditional logistic regression accounting for matching factors only

^b Adjusted for parity, body mass index (BMI), duration of oral contraceptive (OC) use, tubal ligation, smoking status, and family history of ovarian cancer

^c Additionally adjusted for usual sleep duration, snoring habits, and history of rotating night shift work

^d Urinary melatonin levels (aMT6s) are corrected for creatinine concentration. Tertile 1 ranged from -3.76- -1.45; tertile 2 ranged from -1.44- -0.831; tertile 3 ranged from -0.830- 0.59.

^e p-trend was calculated using tertile medians