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Urinary Melatonin in Relation to Breast Cancer Risk: Nested Case–Control Analysis in the DOM Study and Meta-analysis of Prospective Studies

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ABSTRACT

Background: Exposure to higher levels of melatonin may be associated with lower breast cancer risk, but epidemiologic evidence has been limited. We examined the relationship in a case–control study nested within the Diagnostisch Onderzoek Mammacarcinoom (DOM) study and conducted a meta-analysis of prospective studies.

Methods: Concentrations of 6-sulfatoxymelatonin (aMT6s) in prediagnostic first morning urine voids were measured in 274 postmenopausal women diagnosed with breast cancer and 274 matched controls from the DOM study. Conditional logistic regression models were used to estimate multivariable adjusted ORs of breast cancer for thirds of aMT6s. Meta-analysis of this and previous prospective studies of urinary melatonin with breast cancer risk estimated the inverse-variance weighted averages of study-specific log RRs of breast cancer for the highest versus lowest levels of aMT6s.

Results: In the DOM study, the ORs of breast cancer for the middle and highest versus lowest thirds of aMT6s were 0.70 [95% confidence interval (CI), 0.45–1.09] and 0.72 (95% CI, 0.44–1.19), respectively. In the meta-analysis of the DOM study with six previous studies (2,296 cases), RR of breast cancer for the highest versus lowest levels of aMT6s was 0.87 (95% CI, 0.76–1.01).

Conclusions: Results from the DOM study, together with the published prospective data, do not support a strong association of melatonin with breast cancer risk.

Impact: This study adds to the relatively scarce prospective data on melatonin in relation to breast cancer risk. The totality of the prospective evidence does not clearly show an association between melatonin and breast cancer risk, but further data are needed to be able to exclude a modest association.

Introduction

Higher levels of melatonin are hypothesized to be associated with a lower risk of breast cancer (1–10). Night shift work, which probably reduces melatonin production in women, has been categorized by the International Agency for Research on Cancer as probably carcinogenic to humans, mainly based on evidence from animal experiments on the carcinogenicity of alterations of the light–dark schedule (11, 12). Experimental evidence has supported proapoptotic, antiproliferative, antimetastatic, and antiangiogenic actions of melatonin (13).

Of particular relevance to breast cancer, melatonin may interact with estrogen signaling pathways through various mechanisms, for example, inhibiting local synthesis of estrogens by repressing its expression and transactivation and preventing binding of estrogen to estrogen receptor alpha (13). In postmenopausal women, the major source of estradiol is from the conversion of estrone, which is produced primarily in extraglandular tissues from androgen precursors (14);

melatonin may also inhibit the expression and activity of aromatase through regulating gene expression of specific aromatase promotor regions (13).

6-sulfatoxymelatonin (aMT6s), the major metabolite of melatonin, in first morning urine voids is a reliable marker of the nocturnal peak and the total nocturnal output of circulating plasma melatonin (15, 16), which allows epidemiologic investigations into disease risks associated with endogenous melatonin. The most recent meta-analysis, published in 2017, of six cohorts did not find an association between urinary melatonin and breast cancer risk (RR, 0.97; 0.88–1.08; ref. 10). With 1,824 cases and 3,954 controls, however, the data available were relatively limited.

We examined urinary aMT6s levels in relation to risk of breast cancer using a case–control analysis nested within the prospective Diagnostisch Onderzoek Mammacarcinoom (DOM) study. To put the results from the DOM study in the context of the totality of prospective evidence, we conducted a meta-analysis of prospective studies on first morning or 12-hour overnight urinary melatonin levels in relation to breast cancer risk.

Materials and Methods

The DOM study

The DOM study is a population-based, prospective breast cancer screening study conducted in Utrecht and surroundings, the Netherlands. The details of the study have been described elsewhere (17). During 1974–1986, the DOM study recruited 27,718 women, who were born between 1911 and 1945, for mammography screening (18, 19). Mailed self-administrated questionnaires were returned when women attended the physical examinations (20). In addition to mammography screening, physical measurements (e.g., height and weight) were performed at the screening centers and women were asked to donate a first morning urine void on the day of the

examination (19). Urine samples were stored at −20°C in 250 mL polypropylene jars, without preserving agents (19). Urine samples were obtained between 1974 and 1978.

The cases and controls for this study were those who had been selected for a prior investigation of urinary estrogen metabolites and breast cancer risk (21); these were DOM study participants who were postmenopausal women (defined by no menstrual period for at least 12 months, after spontaneous stopping of their menstrual cycles), who did not have a prior history of breast cancer, who were not using exogenous hormones at baseline, who were diagnosed with incident breast cancer at the end of the follow-up period in 1996 (for cases), and who had urine samples available for melatonin analyses. In this sample, 20 women had missing data on melatonin or creatinine, or were excluded because they were no longer in a complete matched set. Women were followed for occurrence of breast cancer until January 1996, through general practitioners, and from 1986 onwards through linkage to the regional cancer registry (19, 22). The initial screenings of the DOM study was carried out at the time when institutional review was not required, but all women gave oral informed consent in accord with the Good Clinical Practice rules at that time (21). The subsequent follow-up study was approved by the institutional review board (20).

Definitions of cases and controls

During follow-up, incident breast cancer cases were identified. Each case was matched to one control according to the following criteria: age at recruitment (within 1 year), date of recruitment (within 1 month), number of donated urine samples (some participants also provided a urine void in the next round of screening), and time interval between urine samples (within 1 month). The analyzed sample included 274 cases and 274 matched controls.

Hormone assays

Assays for aMT6s and creatinine were performed at the laboratories of Stockgrand Limited (University of Surrey, Guildford, England, United Kingdom) in 2016, and case and control participants from the same sets were assayed in the same batch. Urinary aMT6s was assayed in 11 batches using the Bühlmann ELISA (product code EK-M6S; Bühlmann Laboratories AG). Urinary creatinine was assayed in three batches by the Jaffé reaction using the Cayman Creatinine Colorimetric Assay Kit (product code 500701; Cayman Chemical Company). The measurements were done in duplicate and the values for analysis were taken by averaging the two measurements. When urine samples were available from an individual for two timepoints, equal volumes of the samples were mixed to form a pooled aliquot for that individual. This process was applied to both cases and controls within the same matched sets.

Pooled quality controls, produced by combining residual samples from some control participants, were inserted across study samples; in total, 56 quality controls were assayed in duplicate. Laboratory staff were blinded to the status of the samples. The coefficients of variation by batch ranged from 3% to 16% for aMT6s and from 4% to 28% for creatinine.

Statistical analyses

The aMT6s results were reported in ng/mg creatinine (15, 16). Creatinine-adjusted aMT6s levels were divided into thirds using tertile cut-off points in the control participants. Crude summary statistics of personal characteristics were presented by case–control status. The hormone values were logarithmically transformed, and geometric means and 95% confidence intervals (95% CI) of aMT6s levels were estimated for selected categorical personal characteristics in control participants using analyses of covariance, and tests for heterogeneity were assessed. Percentage changes in logarithmically transformed aMT6s levels and 95% CIs per unit change in the continuous variables were estimated by adjusted linear regression and test for trend was assessed by Wald test.

Conditional logistic regression was used to estimate ORs and 95% CIs of breast cancer by thirds of aMT6s levels. On the basis of results from previous studies (10), the regression models were adjusted for the following potential confounders: age at recruitment (continuous), body mass index (kg/m^2; continuous), family history of breast cancer (yes and no), age at menarche (11–12, ≥13 years, and unknown), age at menopause (<45, 45–54, and ≥55 years), parity and age of first birth (nulliparous, 1–2/<25 years, 1–2/≥25 years, ≥3/<25 years, and ≥3/≥25 years), ever use of oral contraceptives (ever and never), ever use of hormone replacement therapy (ever and never), and smoking status (ever and never). We tested for linear trend using a pseudocontinuous variable equal to medians of the thirds of aMT6s levels. As a sensitivity analysis, we excluded cases that were diagnosed within the first 4 years of follow-up, leaving 204 cases and 204 matched controls. Creatinine-adjusted aMT6s levels were redivided into thirds using data from the 204 controls. The analyses were also repeated using the original creatinine-adjusted aMT6s cutoffs.

All statistical analyses were conducted using Stata 15.1 (StataCorp). A two-sided P value of 5% was considered to be statistically significant.

Meta-analysis
Article search and screening

English articles were searched with search terms (Supplementary Table S1) from inception to 22 October 2019 in Embase and MEDLINE. First, duplicates were excluded. Titles and abstracts of studies were screened by two independent reviewers (A.T.Y. Wong and T.Y.N. Tong) according to the following exclusion criteria: (i) the study was not an original study in humans (e.g., systematic reviews, comments, letters, and animal studies), (ii) 12-hour overnight or first morning urinary melatonin was not the exposure (melatonin levels derived from randomly timed spot urine are not informative as a proxy for nocturnal melatonin production; refs. 3, 23), (iii) breast cancer was not the outcome, (iv) the study was not prospective in design, or (v) there was insufficient information (only age-adjusted estimates or published as an abstract only and with insufficient information on the multivariable adjusted estimates, study population, analysis design, or covariate adjustment). In the second stage, full texts were screened again using the same exclusion criteria. Reference lists of the included articles were screened for additional articles. To avoid including participants from the same cohort twice, only data from the analyses with the larger number of cases and/or a longer follow-up period were included for the meta-analysis. This applied to Brown and colleagues (5) and Schernhammer and colleagues (7), in which women were recruited from the Nurses' Health Study II (NHS II) cohort, as well as to Devore and colleagues (6) and Schernhammer and colleagues (8), which analyzed data collected from the NHS cohort. Any disagreement on screening results was resolved by discussion. The flow diagram of the article search is displayed in Supplementary Fig. S1.

Data extraction

The following study characteristics were extracted, wherever available: the first author's surname, study cohort, country, publication year, menopausal status at urine collection, mean age, year of urinary melatonin collection, number of cases and controls, matching criteria,

Table 1. Baseline characteristics of women at recruitment by case–control status in the DOM study.

Characteristics, mean (SD) or number (%) unless specified	Controls (*N* = 274)	Cases (*N* = 274)
Age at baseline (years)	58.5 (3.6)	58.6 (3.6)
Body mass index (kg/m^2)	26.5 (4.3)	26.7 (4.2)
Family history of breast cancer	26 (9%)	45 (16%)
Nulliparous	67 (24%)	50 (18%)
Age at first child (years; among parous women)	28 (5)	27 (4)
Number of children (among parous women)	3.2 (1.9)	3.0 (1.6)
Age at menarche (years)		
11–12	51 (19%)	43 (16%)
13+	92 (34%)	64 (23%)
Not recorded	131 (48%)	167 (61%)
Age at menopause (years)	50 (4)	50 (4)
Ever use of oral contraceptives	7 (3%)	13 (5%)
Ever use of HRT	6 (2%)	7 (3%)
Past or current smoker	65 (24%)	68 (25%)

Abbreviation: HRT, hormone replacement therapy.

type of urine samples, cutoffs or mean levels of the highest and the lowest levels of urinary melatonin, outcome ascertainment, follow-up period, covariates adjusted for, and multivariable adjusted ORs and 95% CIs for breast cancer risk for the highest level versus the lowest level of urinary melatonin. The ORs for breast cancer for the highest third versus lowest third of urinary melatonin were the main results in this study and study by Wang and colleagues (3), and the ORs for the highest fourth and lowest fourth of urinary melatonin were the main results in the remaining included studies.

The Newcastle-Ottawa Scale for cohort studies was used to assess the risk of bias of the included studies on the basis of the design of cohort selection, comparability, and outcome assessment (24).

Statistical analyses

ORs were regarded as RRs on the basis of the rare disease assumption. The overall RR of the meta-analysis was estimated using inverse-variance weighted averages of the logarithmic RR values in the separate studies. Sensitivity analyses were performed with the exclusion of the cases diagnosed soon after baseline, and their matched controls. The *P* values for the Cochran Q test and I^2 estimates were presented for assessing heterogeneity in the estimates across studies. For this sensitivity analysis, it was not possible to include corresponding results for premenopausal women in the ORDET cohort (2) because the analyses were further restricted to participants who also did not smoke. Analyses excluding cases diagnosed in early follow-up were not available for the Guernsey III study (3). Subgroup analysis by menopausal status at urine collection was also performed. The meta-analyses were performed using the "metafor" package (25) and forest plots were generated in R (version 3.5.3, Foundation for Statistical Computing).

Results

The DOM study

Breast cancer cases were diagnosed after a median of 10 years [interquartile range (IQR), 4–15 years]. The median follow-up period for controls was 20 years (IQR, 19–20 years). The mean age at recruitment was 59 years (SD, 4). The median aMT6s levels for breast cancer cases and controls were 28.1 (IQR, 14.8–55.3) and 33.8 (16.9–55.6) ng/mg of creatinine, respectively. The prevalence of family history of breast cancer and younger age at menarche were higher in cases than controls, but other personal characteristics were similar

by case–control status (**Table 1**). Among the controls, women with higher aMT6s levels were more likely to be younger and parous, but were similar to women with lower aMT6s levels with respect to other personal characteristics (**Table 2**).

In the age-adjusted model, ORs (95% CI) of breast cancer for the middle and highest thirds of aMT6s levels, versus the lowest third, were 0.67 (0.45–1.01) and 0.69 (0.44–1.08), respectively (**Table 3**). In the multivariable adjusted models, ORs (95% CI) for the middle and highest thirds versus the lowest third were 0.70 (0.45–1.09) and 0.72 (0.44–1.19; P_{trend} = 0.2), respectively. Findings were similar in sensitivity analyses excluding cases diagnosed in the first 4 years of follow-up [ORs (95% CI) for the middle and the highest thirds of melatonin vs. the lowest third were 0.61 (0.37–1.00) and 0.67 (0.38–1.18) using new cutoffs defined after the exclusion; and 0.62 (0.37–1.03) and 0.67 (0.38–1.19) using the original cutoffs, respectively].

Meta-analysis

We identified 95 articles from the two databases, of which 86 articles were excluded on the basis of duplicates, titles, and abstracts, and a further three articles were excluded after screening for full texts.

Prior to the DOM results, during the past 12 years, six other prospective studies from the United States and Europe were published (Supplementary Table S2). Melatonin data, collected from first morning urine samples (five studies including the DOM study) or 12-hour overnight urine samples (two studies), were available for a total of 2,296 breast cancer cases and 4,494 controls. Women were followed up for invasive breast cancer (two studies) or both invasive breast cancer and *in situ* breast carcinoma (five studies). Most of the studies adjusted for various established risk factors for breast cancer, such as age at menarche, parity, age at first birth, family history of breast cancer, body mass index, and alcohol use. The risk of bias of the included studies was similar and low (Supplementary Table S3).

In the meta-analysis, the summary RR (95% CI) for breast cancer associated with the highest versus the lowest aMT6s levels was 0.87 (0.76–1.01; $P_{\text{heterogeneity}}$ = 0.2; I^2 = 26%; **Fig. 1**). The ORDET study in postmenopausal women (1) also provided an estimate of further adjustment for testosterone in the multivariable adjusted model, and our results were materially unchanged when we included this estimate in the meta-analysis (RR, 0.87; 95% CI, 0.75–1.00).

The sensitivity analysis based on five studies that reported estimates after excluding cases diagnosed in the first few years of follow-up

Table 2. Associations between creatinine-adjusted aMT6s levels (natural logarithmic values) and selected characteristics of control participants in the DOM study, after adjustment for age at urine collection and melatonin and creatinine assay batch, where appropriate.

Characteristics	No. of controls	% change per unit	95% CI	P_{trend}
Age at baseline (years)	274	−3.3	−6.0 to −0.7	0.01
Body mass index (kg/m²)	274	−1.8	−4.0 to 0.5	0.1
Age at menarche (years)	143	−5.0	−12.5 to 2.4	0.2
Age at menopause (years)	274	1.6	−0.9 to 4.0	0.2
Number of children among parous women	207	3.8	−1.6 to 9.2	0.2
Age at first child among parous women (years)	207	−1.4	−3.5 to 0.7	0.2

	No. of controls	Geometric mean, ng/mg creatinine	95% CI	$P_{heterogeneity}$
Family history of breast cancer				
No	248	30.6	27.8–33.8	0.8
Yes	26	29.5	21.7–40.1	
Season of urine collection				
Winter (January–March)	91	27.5	20.2–37.6	0.8
Spring (April–June)	67	28.8	20.2–41.0	
Summer (July–September)	78	35.3	23.9–52.1	
Autumn (October–December)	38	32.3	22.2–47.0	
Parity				
Nulliparous	67	25.1	20.8–30.4	0.02
Parous	207	32.5	29.3–36.2	
Use of oral contraceptives				
Never user	267	30.6	27.9–33.6	NA
Ever user	7	29.2	16.0–53.2	
Use of HRT				
Never user	268	30.4	27.7–33.3	
Ever user	6	39.4	20.9–74.0	NA
Smoking status				
Never smoker	209	30.1	27.1–33.5	0.6
Current or past smoker	65	32.0	26.4–38.8	

Note: Tests for trend of continuous characteristics in relation to aMT6s levels were assessed by Wald test from linear regression, and tests for heterogeneity for categorical characteristics in relation to aMT6s levels were assessed by the global F test from analysis of covariance. NA, test for heterogeneity was not performed owing to the low number of participants in one of the comparison groups.
Abbreviations: HRT, hormone replacement therapy; No., number.

Table 3. Median creatinine-adjusted aMT6s and ORs for breast cancer by thirds of creatinine-adjusted aMT6s in 274 women who developed breast cancer and 274 matched control participants from the DOM study.

|	Third of creatinine-adjusted aMT6s		
	Lowest (<22.6 ng/mg)	Middle (22.6–46.2 ng/mg)	Highest (>46.2 ng/mg)
Median aMT6s[a] (IQR)			
Cases	13.6 (8.6–18.3)	32.7 (27.9–36.4)	70.7 (56.6–106.7)
Controls	12.9 (7.9–17.0)	34.2 (27.7–40.0)	68.5 (55.6–100.2)
OR (95% CI) for breast cancer			
Age-adjusted	1.00 (—)	0.67 (0.45–1.01)	0.69 (0.44–1.08)
Multivariable adjusted[b]	1.00 (—)	0.70 (0.45–1.09)	0.72 (0.44–1.19)
Multivariable adjusted, follow-up ≥4 years[c]	1.00 (—)	0.62 (0.37–1.03)	0.67 (0.38–1.19)
Multivariable adjusted, follow-up ≥4 years[d]	1.00 (—)	0.61 (0.37–1.00)	0.67 (0.38–1.18)

[a]Creatinine-adjusted aMT6s.
[b]Adjusted for age at recruitment, body mass index, family history of breast cancer, age at menarche, age at menopause, parity and age of first birth, ever use of oral contraceptives, ever use of hormone replacement therapy, and smoking status.
[c]On the basis of 204 matched case–control sets and with thirds of creatinine-adjusted aMT6s defined using cut-off points from the main analysis.
[d]On the basis of 204 matched case–control sets and with thirds of creatinine-adjusted aMT6s defined using cut-off points on the basis of tertiles in controls from match sets with cases diagnosed ≥4 years after blood collection. Cut-off points for the middle and the highest thirds were 24.2 and 46.9 ng/mg creatinine, respectively.
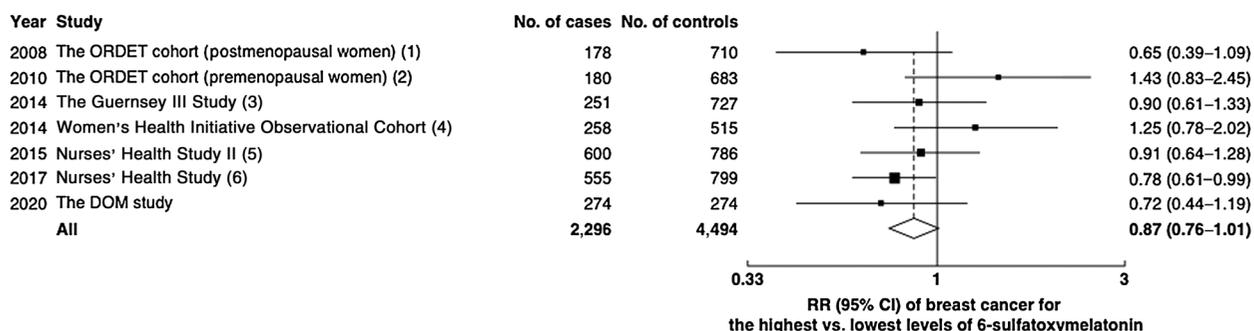
Figure 1.
Meta-analysis of seven prospective studies on risk of breast cancer for the highest versus the lowest level of urinary aMT6s. RRs of the individual studies are presented as squares with lines corresponding to the 95% CIs. Sizes of the squares are inversely proportional to the variance of the study-specific logarithmic RRs. The diamond represents the overall RR. $P_{\text{heterogeneity}} = 0.2$; $I^2 = 26$. No., number.

showed similar findings [RR, 0.88 (0.69–1.13); $P_{\text{heterogeneity}} = 0.08$; $I^2 = 53\%$; **Fig. 2**). Six studies provided estimates separately for premenopausal and/or postmenopausal women at baseline, and we found no evidence of heterogeneity in the association of breast cancer risk with urinary melatonin by menopausal status (test for heterogeneity, $P = 0.2$).

Discussion

In this study, nested within the DOM cohort, levels of melatonin in first morning urine samples were not associated with the risk of breast cancer in postmenopausal women, and our meta-analysis of seven prospective studies, including the DOM study, does not support an overall association of breast cancer risk with 12-hour overnight or first morning urinary melatonin level in women.

Compared with the most recently published meta-analysis, in 2017 (10), this meta-analysis includes the new results from the DOM cohort, and the updated results with extended follow-up from the NHS cohort (6). In total, 1,824 cases were included in the previous meta-analysis (10), while 2,296 cases were included in this meta-analysis. These relatively scarce published data somewhat limit the conclusions that can be drawn and we were unable to exclude a modest association between urinary melatonin and breast cancer risk. Further prospective

data are needed, but are difficult to generate because only a few large cohort studies have the appropriate baseline urine samples. Other epidemiologic approaches, such as Mendelian randomization analyses, if appropriate genetic instruments can be identified, may be informative.

To evaluate potential reverse causation bias, we restricted the meta-analysis to five studies that excluded cases diagnosed soon after baseline and observed similar null findings. Although the $P_{\text{heterogeneity}}$ was not statistically significant, the amount of heterogeneity quantified in I^2 was moderate (53%). Some differences in the study designs may explain the moderate heterogeneity in this sensitivity analysis. There were differences in the numbers of years that were excluded in the sensitivity analyses for each individual study (i.e., 4–6 years), and the ORs appeared to be closer to the null hypothesis for studies with a longer period of exclusion. To allow for better examination of whether time to diagnosis is relevant to the associations of melatonin with risk of breast cancer, future collaborative reanalyzes using individual-level data from published prospective studies are required (26). Also, while the ORDET study for postmenopausal women collected 12-hour overnight urine samples, the rest of the studies collected first morning urine voids, but we would not expect a large difference in the measurements as most of the production of melatonin occurs during the biological night. Moreover, there was reduced power to detect or
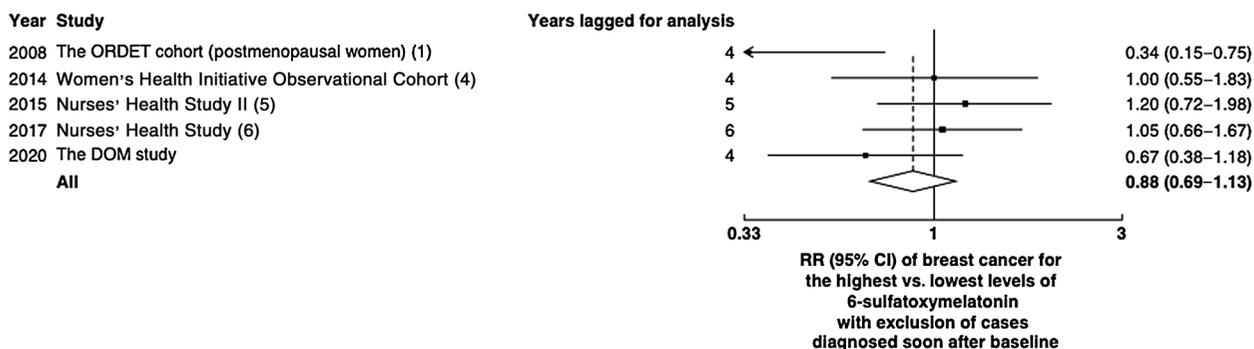
Figure 2.
Sensitivity analysis of the meta-analysis of risk of breast cancer for the highest versus the lowest level of urinary aMT6s from five prospective studies, in which breast cancer cases diagnosed within early periods of follow-up were excluded. RRs from the individual studies are presented as squares with lines corresponding to the 95% CIs. Sizes of the squares are inversely proportional to the variance of the study-specific logarithmic RRs. The diamond represents the overall RR. $P_{\text{heterogeneity}} = 0.08$; $I^2 = 53\%$.

refute an association in the analyses that excluded cases diagnosed in early follow-up. Hence, the results in the analyses that excluded cases diagnosed in early follow-up would be more prone to more extreme values owing to sampling variation.

Possible associations of demographic and lifestyle factors with endogenous melatonin levels remain unclear. Unexpectedly, the proportion of nulliparous women was higher among controls than cases, but this could be due to chance. Among the control participants in the DOM study, we found an inverse association of aMT6s levels with age consistent with previous studies (3, 27). We also observed a higher aMT6s level in parous than nulliparous women, which has also been found in a previous NHS analysis (28), although not in another similar analysis (3). It is not clear why melatonin levels should be higher in parous women and this may be a chance finding. We found no evidence for an association of melatonin levels with other reproductive factors. However, we did not find any significant association of aMT6s levels with body mass index (27, 28) or smoking status (28), although such associations have been reported in some previous studies.

Putative mechanisms linking melatonin and breast cancer risk involve steroid hormones, and hence, the melatonin–breast cancer relationship may differ between premenopausal and postmenopausal women potentially via interaction with ovarian hormones (9). In this meta-analysis, we did not find significant heterogeneity by menopausal status, but there was limited power for this comparison, and insufficient data in the included studies for us to evaluate possible heterogeneity by tumor receptor status.

Our use of first morning urinary samples is a valid surrogate for nocturnal melatonin production (15, 16, 29). Previous studies demonstrated high correlation of aMT6s levels between 2 days of measurements (30), and reported good reproducibility of first morning urinary melatonin levels over a 3-year period in premenopausal women (31) and over a 5-year period in postmenopausal women (32). Such reproducibility is comparable with that of other plasma hormones, such as estradiol and estrone, over a 2- to 3-year period (33).

There were some limitations of the analyses in the DOM study and the meta-analyses. In the DOM study, we were unable to adjust for alcohol intake, personal history of benign breast disease, use of beta-blockers, calcium channel blockers, or psychotropic medications (27), or shift or night work status (31), as those data were not available. However, we note that in a subsequent study of women for a breast cancer screening project in the same region, the prevalence of alcohol consumption in postmenopausal women was found to be low (34). There is no consistent and strong cross-sectional evidence for the associations of melatonin with alcohol consumption and antidepressant use (27, 28, 35), so it is unlikely that our results would be largely confounded by these factors. In the meta-analyses, the ratios of the highest over the lowest cutoffs for aMT6s varied across studies, ranging from 2-fold to nearly 4-fold. However, our meta-analysis aimed to compare the top and bottom ends of the distribution of aMT6s in relation to breast cancer risk, which only assumes that the distributions, not the actual concentrations, of hormone levels were similar across studies. Finally, we cannot exclude the possibility that other parameters of melatonin production, such as the difference between the nocturnal peak level and daytime level, would be associated with breast cancer risk (9).

To conclude, this prospective evidence does not support a strong association between urinary melatonin levels and breast cancer risk. However, the published observational data remain scarce, limiting the conclusions that can be drawn. Further prospective studies, as well as meta-analyses, together with other epidemiologic approaches, such as Mendelian randomization analyses, are required to clarify the association.

Authors' Disclosures

Authors' Contributions

A.T.Y. Wong: Formal analysis, funding acquisition, investigation, methodology, writing–original draft, writing–review and editing. G.K. Fensom: Data curation, investigation, writing–review and editing. T.J. Key: Conceptualization, project administration, writing–review and editing. N.C. Onland-Moret: Conceptualization, resources, data curation, writing–review and editing. T.Y.N. Tong: Supervision, methodology, writing–review and editing. R.C. Travis: Conceptualization, resources, supervision, funding acquisition, methodology, project administration, writing–review and editing.

Acknowledgments

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