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A cross-sectional study of breast cancer biomarkers among shift working nurses

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ABSTRACT

Objectives: In 2007, the International Agency for Research on Cancer classified long-term shift work as a probable carcinogen, with the strongest evidence for breast cancer. One proposed mechanism involves night-time light exposure and decreases in melatonin, a circadian rhythmic hormone. It is hypothesised that melatonin influences patterns of sex hormone production that in turn influence breast cancer risk. This study sought to investigate the relationships of shift work history, 6-sulfatoxymelatonin (aMTs-6, the primary melatonin metabolite) and sex hormone levels among shift working nurses.

Design: This is a cross-sectional biomarker study.

Setting: 94 premenopausal nurses who work a full-time rotating shift schedule at one Ontario hospital were recruited for this study; 82 completed follow-up.

Primary and secondary outcome measures: Study participants provided morning void urine and fasting blood samples for the assessment of aMTs-6 and sex hormone (oestradiol, oestrone, progesterone, prolactin) levels, respectively. These data were collected at two time points (summer and winter) such that relationships between melatonin and sex hormones could be assessed with respect to two time frames of interest (acute and cross-seasonal).

Results: An inverse relationship between aMTs-6 and oestradiol was suggested in the winter ($\beta=-0.18$, $p=0.04$), but this result was not statistically significant in multivariate modelling that adjusted for age, body mass index and menstrual cycle. Likewise, while oestradiol, oestrone and progesterone levels increased with greater years of shift work history (all $p<0.05$), these associations were attenuated after confounder adjustment.

Conclusions: These results do not support the proposed relationship between melatonin and sex hormone levels as biomarkers on the pathway of shift work and breast cancer but emphasise the importance of adjusting for confounders in modelling.

ARTICLE SUMMARY

Article focus

- Melatonin and sex hormones are proposed intermediates in the pathway of shift work to a possible increased breast cancer risk.
- This hypothesis is based on associations between shift work, exposure to light at night and reduced melatonin levels; between reduced melatonin and increased sex hormone levels and between increased sex hormone levels and increased breast cancer risk.
- Few studies have investigated relationships between melatonin and sex hormone levels in an observational setting, which is of relevance to pathways to carcinogenesis, and many studies are limited by uncontrolled confounding.

Key messages

- The results of this study do not support hypothesised relationships between shift work, melatonin and sex hormone levels as intermediates in the pathway to breast cancer but highlight the importance of confounder adjustments, which has not been adequately addressed in much previous work.

Strengths and limitations of this study

- This study was restricted to women who work one rotating shift work pattern, which may not be sufficiently disruptive to circadian rhythms to cause measureable changes in melatonin and/or sex hormone levels.
- While this may contribute to the lack of associations observed, it may suggest very good news to women working this prevalent rotating shift pattern.
- The study was limited by a small sample size and was not sufficiently powered to detect small (<0.2) associations between melatonin and sex hormones.

INTRODUCTION

As shift work has become essential to our modern 24-h society, researchers and workplace compensation boards are increasingly concerned of possible health effects.[1] The aetiological role of this prevalent occupational exposure has been investigated in numerous health conditions from sleep disturbances and social well-being to major chronic diseases, such as heart disease, metabolic syndrome and cancer.[2 3] Based on an accumulation of epidemiological and experimental evidence, shift work was

formally classified as a probable carcinogen by the International Agency for Research on Cancer in 2007, with the strongest support provided for breast cancer.[4] While meta-analyses have suggested that shift working women may be at 40%–50% increased risk of breast cancer[5 6]; individual studies are inconsistent in their findings and hence the evidence for this association is still limited.[7] While future research of cancer risk among shift workers is necessary, biomarker studies that investigate possible intermediates of this proposed relationship are desired to help clarify the plausibility of this link.[8]

One hypothesis for the association between shift work and breast cancer involves exposure to light at night and subsequent disturbances to the body's normal circadian (24-h) rhythms.[1 6 7] This is highly plausible given that melatonin, a hormone whose secretion by the pineal gland is stimulated by darkness and inhibited by light exposure, has demonstrated cancer-protective effects.[9] In line with most known breast cancer risk factors, melatonin may interact with sex hormone levels that in turn regulate the development and progression of breast tumours.[10–12] In sum, it is postulated that exposure to light at night during shift work suppresses melatonin production leading to increases in sex hormones, which may in turn increase breast cancer risk.

Relationships between melatonin and sex hormone levels are well known in seasonally breeding animals whose melatonin secretion, influenced by seasonal daylight patterns, controls seasonal mating through alterations in oestrogen levels.[13] In humans, relationships between melatonin and sex hormones have been identified at menarche and menopause and in certain pathological conditions, such as anovulation and oophorectomy.[14] In addition, significant reductions in sex hormone levels have been demonstrated in randomised controlled trials of melatonin supplementation.[15–18] Despite these findings, there are very limited and inconsistent research findings to indicate the presence of relationships between melatonin and sex hormones in healthy women under a normal physiological setting, which are relevant to pathways in carcinogenesis.[18–22] The purpose of this study was to examine the association between melatonin, sex hormone levels and shift work history among healthy premenopausal nurses, as potential intermediates in the pathway to breast cancer.

MATERIALS AND METHODS

Study population

Following institutional research ethics board approval, 94 cancer-free premenopausal nurses at Kingston General Hospital (KGH) consented to participate in this cross-sectional biomarker study. Recruitment took place from April 2008 to February 2009 through posters and pamphlets that were sent to all full-time nurses at the hospital. Women self-excluded if they did not perform shift work (which is a rotating pattern of two 12-h day shifts, followed by two 12-h night shifts, followed by 5 days off at this hospital), if they had been pregnant or lactating in the previous 6 months or if they used melatonin supplements. Of the 94 participants recruited, seven withdrew from the study and five did not provide blood samples and so were excluded. Eighty-two women (87%) comprised the final study sample.

Data collection

Upon study enrolment, participants completed a questionnaire to gather health, lifestyle and employment information. Prior to commencing a day shift of their regular rotating shift pattern (05:00–07:00), nurses provided morning void urine and fasting blood samples. At this time, nurses completed a diary to record their current menstrual cycle stage (follicular (days 0–10), mid-cycle (days 11–16) and luteal (day 17+), based on cyclical changes in oestrogen and progesterone levels over the cycle), current oral contraceptive use, typical physical activity levels of the past month and daily caffeine consumption.

These data collection were completed at two time points, in summer and winter, to investigate two hypothesised time frames of interest for associations of melatonin and sex hormone levels, namely (1): immediate (acute) relationships and (2) latent relationships across seasons. This latter analysis was conducted based on a hypothesised latency period for influences of altered melatonin levels on sex hormones, as demonstrated from melatonin trials that show significant reductions in sex hormone levels after 4–6 months of supplementation.[15 16 18] Sixty-eight of the 82 nurses enrolled participated in the summer study period and 65 in winter, with 51 women completing both study periods and 31 participating once.

Melatonin assessment

Melatonin levels were characterised by concentrations of the primary urinary melatonin metabolite, 6-sulfatoxymelatonin (aMTs-6), measured from first morning void urine samples. Urinary aMTs-6 levels correlate well with circulating melatonin levels in blood, and aMTs-6 concentration in first morning void urine accurately characterises the peak nocturnal melatonin secretion as well as the total nocturnal melatonin output (accounting for approximately 70% of total circulating melatonin from the previous night).[23]

Nocturnal melatonin secretion is relatively stable among individuals; aMTs-6 assessment from a single morning void urine sample has been found to be highly reproducible over 3 to 5 years.[19 24] Pilot work in this study population validates the accuracy of melatonin assessment from morning void urine, as the timing of peak melatonin production is not altered (it still occurs at night) among nurses working this rotating shift schedule.[25]

Prior to commencing their shift, nurses collected their first morning void urine (at 05:00–07:00) and delivered samples to the KGH core laboratory. Urine samples were processed, aliquoted and stored at −80°C until

melatonin analysis. Concentrations of aMTs-6 were assayed using the Bühlmann aMTs-6 ELISA (ALPCO, Salem, New Hampshire, USA) and were adjusted for creatinine levels (aMTs-6 divided by creatinine concentration, in nanograms per milligram) to account for variability in the diluteness of urine samples. Creatinine concentrations were measured from the same urine samples using the Parameter Creatinine Assay (R&D Systems, Minneapolis, Minnesota, USA).

All urine samples were analysed in duplicate for aMTs-6 and creatinine concentrations and an average concentration and coefficient of variation (CV) were calculated for each sample. Median CVs for aMTs-6 and creatinine were 9.1% and 10.0%, respectively. To ensure data quality, assays for which the standard curve poorly fit the sample standards (R^2 <0.95) were repeated. Additionally, samples for which aMTs-6 or creatinine concentrations were out of range of the assay's standard curve were re-analysed using appropriately adjusted dilutions.

Sex hormone assessment

Concentrations of oestradiol (in picomoles per litre), oestrone (in picomoles per litre), progesterone (in nanomoles per litre) and prolactin (in micrograms per litre) were measured by immunoassay from fasting blood samples that were taken on the same day and at the same time (05:00–07:00) as urine samples. Serum levels of progesterone, oestradiol and prolactin were measured at the KGH core laboratory, and serum oestrone levels were measured at the McMaster University Medical Centre. Blood samples were analysed immediately upon retrieval at the hospital core laboratory, and blood for oestrone analysis was processed, aliquoted and frozen prior to shipment to the McMaster University Medical Centre. Immunoassay tools were calibrated at each use, and all samples were analysed with standard controls. Inter-assay precision (CV) of these analyses were all <20%.

Statistical analysis

Natural logarithms of aMTs-6 and sex hormones were used for analysis as transformed values more closely approximated normal distributions. Generalised linear regression modelling was used to examine relationships between melatonin and each sex hormone. Relationships were also examined separately by season, as well as across seasons whereby melatonin levels from the first season were assessed in relation to sex hormone levels from the second season. For all regression models, a change-in-estimate selection procedure (≥10% change in the main exposure effect) was used to identify confounders,[26] while age, body mass index (BMI) and menstrual cycle stage were included in all final models. Other variables considered potential confounders a priori included current oral contraceptive use (yes/no), average physical activity levels (including intensity, frequency and duration; conceptualised in hours per week for all intensities and for moderate-vigorous intensity activity only), alcohol consumption (average drinks per week), caffeine consumption (average drinks per week) and smoking status (current, former, never). All statistical analyses were completed using SAS (V.9.2; SAS Institute).

RESULTS

Characteristics of the study population are presented in tables 1 and 2. The majority of nurses had worked night shifts for at least 5 years. While many women were either overweight or obese, most women were non-smokers and did not use oral contraceptives.

No associations were observed between melatonin (conceptualised as a continuous variable and by tertiles) and sex hormones (table 3, figure 1). While an inverse relationship between melatonin and oestradiol was suggested in winter (β=−0.18, p=0.04), this result was not statistically significant after accounting for the confounding influences of age, BMI, menstrual cycle stage, oral contraceptive use and recent alcohol consumption. On investigation of a longer latency period, melatonin levels in the first season were not associated with oestradiol or oestrone levels in the second season. No relationship was observed between melatonin and progesterone or prolactin in crude and adjusted analyses.

Sensitivity analysis revealed that results were unchanged with the exclusion of oral contraceptive users, of nurses who provided blood samples over 1 h past the prescribed time frame, and when non-transformed variables were used for analyses.

Associations between shift work history (conceptualised as continuous years and <20 vs 20+ years) and sex hormones are presented in table 4. While statistically significant associations were observed between

Table 1 Baseline characteristics of the study population (n=82)

Characteristic	N (%)
Personal	
Age (years), mean±SD	35.8±8.2
Body mass index (kg/m^2), mean±SD	27.1±6.7
Ethnicity	
White	79 (96)
Non-white	3 (4)
Lifestyle	
Smoking	
Smoker	11 (13)
Past-smoker	19 (23)
Never-smoker	52 (63)
Alcohol consumption (drinks per week), mean±SD	3.4±3.8
Employment	
History of shift work (years), mean±SD	11.0±8.4
≤5	22 (27)
>5–10	28 (34)
>10–15	9 (11)
>15–20	8 (10)
>20	15 (18)

Table 2 Seasonal characteristics of the study population

Characteristic	Summer (n=68), N (%)	Winter (n=65), N (%)
Reproductive		
Menstrual cycle stage		
Follicular (days 1–10)	26 (38)	22 (34)
Mid-cycle (days 11–16)	10 (15)	9 (14)
Luteal (day 17+)	28 (41)	32 (49)
Current OC use		
Yes	13 (19)	12 (19)
No	53 (78)	51 (79)
Lifestyle		
Total physical activity (all intensities) (hours per week), mean±SD	4.4±4.2	4.7±5.5
Moderate-to-heavy physical activity* (hours per week), mean±SD	3.3±3.7	3.6±5.4
Caffeinated beverage consumption (drinks per day), mean±SD	3.3±3.3	3.0±2.7

Percentages may not add due to rounding and/or missing covariate information (menstrual cycle stage, n=4; OC use, n=2).
*Increases the heart rate and breathing rate and may cause sweating.
OC, oral contraceptive.

continuous years of shift work and each of oestradiol, oestrone and progesterone levels (all ps<0.05), these results were confounded, particularly by age, and were not statistically significant after confounder adjustment. Similarly, while geometric means of oestradiol, oestrone and progesterone were consistently higher among longer-term shift workers (<20 vs 20+ years), multivariate-adjusted differences were not statistically significant.

DISCUSSION

Overall, this study found no evidence for an association between melatonin, shift work history and sex hormone levels. While some relationships were observed in crude analyses, results were not statistically significant after confounder adjustment. Previous studies have reported statistically significant inverse relationships between melatonin and follicle-stimulating hormone (r =−0.322, p<0.05), luteinising hormone (r=−0.314, p<0.05)[18] and oestradiol (r=−0.661, p<0.001)[21] in similar observational settings, but these results may be attributed to uncontrolled confounding as the results were not adjusted for important factors such as age and BMI.

The results of more recent observational studies with comprehensive confounder assessment are consistent with our findings. Two studies investigated relationships between melatonin and sex hormones in women of the Nurses' Health Study cohort.[19 20] Among 80 premenopausal nurses who did not use oral contraceptives, bivariate associations found between melatonin and each of bioavailable oestradiol and progesterone were no longer statistically significant after adjustment for age and BMI.[19] Furthermore, while a longer duration of shift work (15 years or more) was associated with increased levels oestradiol (p=0.03), this result was confounded and no longer statistically significant in multivariate modelling.[19] The investigation of a larger group of women (n=459) in this cohort revealed no association between melatonin and sex hormone levels.[20] A more recent study of 206 postmenopausal Japanese women also found no evidence for a relationship between melatonin and sex hormones, yet having 'ever worked night shifts' was related to increased oestrone levels (p<0.01).[22] In sum, the results of our study are consistent with these recent observational studies, which show no strong relationships between melatonin and sex hormones while accounting for confounding variables.

Despite limited observational evidence, some support for these relationships has been reported under certain pathological or pharmacological conditions. Unusually high melatonin levels have been associated with anovulation, oophorectomy and other abnormal ovarian conditions,[14] and randomised trials of melatonin supplementation have shown reductions in oestrogen, luteinising hormone, follicle-stimulating hormone and inhibition of ovulation with pharmacologic doses of melatonin.[15–18] If the proposed relationships exist, it is possible that under extreme or unnatural conditions

Table 3 Multivariate-adjusted associations between creatinine-adjusted 6-sulfatoxymelatonin (in nanograms per milligram) and sex hormones (log-transformed)

	Analysis		
Sex hormone	Summer Parameter estimate (p value)	Winter Parameter estimate (p value)	Across Seasons Parameter estimate (p value)
Oestradiol (pmol/l)	0.005 (0.94)	−0.13 (0.11)	−0.05 (0.54)
Oestrone (pmol/l)	0.05 (0.36)	−0.03 (0.61)	0.05 (0.40)
Progesterone (nmol/l)	0.07 (0.46)	0.14 (0.22)	−0.02 (0.86)
Prolactin (μg/l)	0.01 (0.78)	0.003 (0.94)	−0.05 (0.40)

Adjusted for age (years), body mass index (kg/m^2), menstrual cycle stage (follicular, mid-cycle, luteal) and OC use (yes/no), recent alcohol consumption (drinks per day), recent caffeine consumption (drinks per day), smoking status (current/never/former) and total and moderate-to-heavy recent physical activity levels (hours per week), as appropriate.

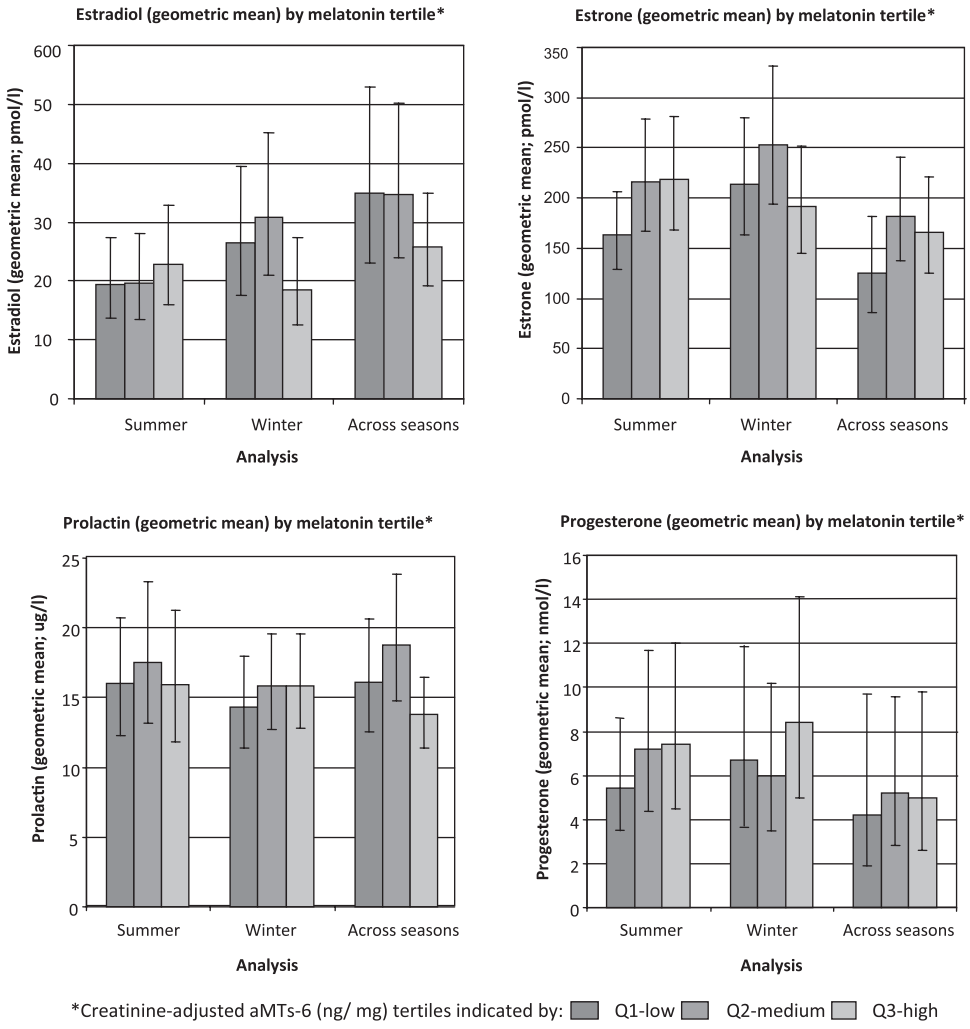

Figure 1 Multivariate-adjusted geometric means of sex hormones by tertile of creatinine-adjusted 6-sulfatoxymelatonin (aMTs-6) (in nanograms per milligram).

such as these the relationships are more apparent. In the same way, these relationships could be more obvious in shift workers who experience greater disruptions to their melatonin secretion, such as permanent night workers or those with a longer history of shift work. Among our rotating shift nurses, it is possible that their work schedule of two 12-h days, followed by two 12-h nights, followed by 5 days off is not sufficiently disruptive to circadian rhythms to cause such changes in melatonin and/or sex hormone levels. This could be very good news to women working this prevalent shift pattern.

The results of this study must be considered in light of the small sample size. Our study was sufficiently powered (at 80% power and α=0.05) to detect effect estimates in the range of ±0.2 or greater. Therefore, these results alone are unable to refute the possibility of small (<0.2) relationships between melatonin and sex hormones.

This study contributes observational evidence concerning the relationships between melatonin and sex hormones as potential intermediates of shift work and breast cancer, an area with few existing studies that have had mostly null findings.[19 20 22] Given the limitations in

Table 4 Multivariate-adjusted associations between night shift work (continuous years; <20 vs 20+ years) and sex hormones (log-transformed)

Sex hormone	Continuous shift work (years) Parameter estimate (p value)	Dichotomous shift work (<20 vs 20+ years), geometric means (95% CI)		
		<20 years (n=67)	20+ years (n=15)	p for Diff
Oestradiol (pmol/l)	−0.002 (0.87)	259.4 (198.4–338.9)	269.5 (182.6–397.7)	0.84
Oestrone (pmol/l)	0.005 (0.48)	209.1 (174.3–250.8)	258.6 (197.9–337.9)	0.11
Progesterone (nmol/l)	−0.002 (0.86)	7.0 (4.60–10.8)	7.5 (4.28–13.2)	0.79
Prolactin (μg/l)	0.002 (0.79)	16.5 (13.4–20.2)	15.7 (11.8–20.8)	0.71

Adjusted for age (years), body mass index (kg/m^2), menstrual cycle stage (follicular, mid-cycle, luteal) and OC use (yes/no), recent alcohol consumption (drinks per day), recent caffeine consumption (drinks per day), smoking status (current/never/former) and total and moderate-to-heavy recent physical activity levels (hours per week), as appropriate.

research on shift work and breast cancer to date, for which evidence is yet conclusive,[7] the investigation of potential intermediates may help clarify the plausibility of this link. Furthermore, an identification of the intermediate pathways is crucial to the development of strategies to minimise the health impacts of this work schedule. Future studies of ideally larger sample sizes may consider investigating these relationships among women working more extreme shift schedules or among longer-term shift workers, as these groups may be more likely to experience biological changes that lead to detectable disruptions of hormone levels.

The results from this study alone cannot rule out these proposed relationships between melatonin and sex hormones nor other potential mechanisms to explain increases in breast cancer incidence among shift workers. Future studies should investigate other proposed intermediates of this pathway, including melatonin's antioxidant actions and immunomodulatory functions.[13] In addition, several alternative pathways outside the melatonin hypothesis should be considered including behavioural or lifestyle changes and sleep disturbances[27] as these changes may also help explain increases in breast cancer risk among shift workers.

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Competing interests None.

Ethics approval Ethics approval was provided by Queen's University Research Ethics Board.

Contributors This manuscript presents results from an observational study ('Occupational and other factors as determinants of melatonin levels among rotating shift nurses') lead by Dr KJ Aronson (PI) and co-investigators Drs H Richardson, JE Tranmer, CH Graham, I Janssen and G Jones. The investigation of sex hormone levels among these nurses was completed as a substudy to the original work, lead by Dr KJ Aronson, AR Langley and A Grundy. ARL completed the statistical analyses, interpretation of results and manuscript writing with editorial support and intellectual input from all co-authors. All authors approved the final version of this manuscript.

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