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Research Article

Rotating Night Shift Work and Mammographic Density

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Abstract

Background: An increased risk of breast cancer has been observed in night shift workers. Exposure to artificial light at night and disruption of the endogenous circadian rhythm with suppression of the melatonin synthesis have been suggested mechanisms. We investigated the hypothesis that rotating night shift work is associated with mammographic density.

Methods: We conducted a cross-sectional study on the association between rotating night shift work characteristics, 6-sulfatoxymelatonin (MT6s) creatinine adjusted in a spot morning urine sample, and a computer-assisted measure of mammographic density in 640 nurses and midwives ages 40 to 60 years. The associations were evaluated using regression models adjusted for age, body mass index, menopausal status, age at menopause, age at menarche, smoking, and the calendar season of the year when mammography was conducted.

Results: The adjusted means of percentage of mammographic density and absolute density were slightly higher among women working rotating night shifts but not statistically significant [percentage of mammographic density = 23.6%, 95% confidence interval (CI), 21.9%–25.4% vs. 22.5%, 95% CI, 20.8%–24.3%; absolute density = 23.9 cm^2, 95% CI, 21.4–26.4 cm^2 vs. 21.8 cm^2, 95% CI, 19.4–24.3 cm^2 in rotating night shift and day shift nurses, respectively). There were no significant associations between the current or cumulative rotating night shift work exposure metrics and mammographic density. No association was observed between morning MT6s and mammographic density.

Conclusions: The hypothesis on the link between rotating night shift work, melatonin synthesis disruption, and mammographic density is not supported by the results of the present study.

Impact: It is unlikely that the development of breast cancer in nurses working rotating night shifts is mediated by an increase in mammographic density. *Cancer Epidemiol Biomarkers Prev; 21(7); 1028–37. ©2012 AACR.*

Introduction

Night shift work has been classified by the International Agency for Research on Cancer (Lyon, France) as a probable carcinogen (1). This classification was based on the evidence from experimental studies on animals that showed carcinogenicity of artificial light during the dark period (biologic night), combined with limited evidence from studies in humans on cancer risks associated with shift work that involves night work. Eight (2–9) of 12 epidemiologic studies (2–13) that have addressed this topic have reported a statistically significant association between night shift work and the risk of breast cancer. The biologic mechanisms underlying the increased breast cancer risk in night shift workers have not been fully recognized yet, nevertheless, sleep deprivation, disruption of the circadian rhythm, dysregulation of the circadian rhythm genes, and inhibition of melatonin synthesis have been suggested as potential explanations (14–16). An inverse association between melatonin and breast cancer risk has been observed in some (17–20), but not all, prospective epidemiologic studies (17–21).

Melatonin, a pineal gland hormone, has been found to act as an inhibitor of tumor cell proliferation in animal models and in human cancer cells lines (22). Its anticarcinogenic properties have been explained by its antiestrogenic and antioxidative activity, stimulation of the immune system, and its role in the cell-cycle regulation (23).

Mammographic density reflects the tissue composition of the breast and is positively associated with the amount of collagen and the number of epithelial and nonepithelial cells and negatively associated with fat (24). It has been found to be a strong and independent risk factor of breast cancer, with a 4.6-fold increased breast cancer risk

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observed in women with extensive (>75%) percentage of mammographic density, when compared with women with a small proportion of dense areas in the breast (<5%; ref. 25).

Given the observations of an increased risk of breast cancer in night shift workers, it could be hypothesized that prolonged night shift work, through melatonin-related mechanisms or other mechanisms, may possibly influence mammographic density. To test this hypothesis, we set up a cross-sectional study examining the association between night shift work history and mammographic density in nurses. In addition, we tested the association between 6-sulfatoxymelatonin (MT6s; melatonin metabolite), as a biomarker of circadian rhythm disruption, and mammographic density.

Materials and Methods

This cross-sectional study included nurses and midwives registered at the Local Registry of the Chamber of Nurses and Midwives in Lodz, Poland. A total of 1,117 nurses and midwives was randomly selected for the study, based on the registry database (i.e., ~30% of the registered in this age group), and of these, 924 (83%) were contacted. Inclusion criteria (age 40–60 and current employment as a nurse in public health care setting in Lodz) were confirmed for 866 women, and 725 (84% of the eligible subjects) women agreed to participate in the study.

A structured questionnaire was administered during in-person interviews, and information was obtained on occupational history, demographics, medical and reproductive history, hormone use, physical activity (according to the international questionnaire on physical activity, IPAQ; ref. 26), smoking and alcohol use, diet, and sleep quality (according to Pittsburg Sleep Quality Questionnaire; ref. 27). Body weight and height, hip and waist circumferences were measured.

Of 725 women who agreed to participate in the study, 681 (i.e., 95%) had undergone screening mammogram. As many as 41 took hormonal medications for menopause symptoms at the time of the mammography and were excluded, leaving 640 women for the analysis.

Digital mammography was conducted with MAMMOMAT Novation DR, Siemens, Munich, the Federal Republic of Germany, according to the standard procedure in the Center of Laser Diagnostics and Treatment of the Technical University in Lodz. The examination of the breasts included both craniocaudal and mediolateral oblique views. The mean time between urine collection and mammography was 130 days, and in 462 women (72%), mammographic examination was conducted within 6 months of the interview.

The study was approved by the Ethical Institutional Review Board at the Nofer Institute of Occupational Medicine (Lodz, Poland). A signed informed consent was obtained from each study participant.

Mammographic density assessment

Left breast craniocaudal views were used for the mammographic density assessment. The images were transformed to .jpg files using the ImageMagick Program (Magick Studio LLC) with the resolution of 832 × 1,021 pixels. The University of Southern California (Los Angeles, CA) Madena computer program was used to determine mammographic density. The method has been previously described and validated (28, 29). The total dense area − absolute density (in cm^2) and percentage of density (calculated as the ratio of the dense area to the total breast area) were estimated. The percentage of mammographic density, or the fraction of the breast with densities, is the ratio of absolute density to the total breast area. The density assessments were conducted by G. Ursin and the outline assessments by A. Bukowska.

For the reproducibility assessment, the total breast area and the dense areas readings were repeated for 10% of the images, randomly selected from the database, ensuring blindness of the readers in respect of the subjects' identifiers as well as the previous readings results. The test-retest estimates were highly correlated, with $R = 0.98$ ($P <$ 0.00001) for both the total breast area and the dense areas estimates.

Breast mammograms were also examined by radiologists who classified the images according to the BI-RADS classifications (30). A comparison of the 2 methods was carried out with Kendall correlation test, resulting with $\tau = 0.69$ ($P < 0.0001$).

Urine samples collection and MT6s analysis

The procedures of urine sample collection and analyses have been described previously (31). In short, the participants provided a spot morning urine sample (6–8 am). In the case of night shift workers, the urine sample was taken at the end of the night shift. The samples were collected by trained nurses and delivered to the laboratory immediately. They were frozen at −20°C in the laboratory and stored until analysis. Immediately before the analysis, thawed samples were centrifuged, and the supernatant was used for MT6s determination.

We analyzed MT6s using an ELISA test (DRG International, Inc.). The MT6s levels were creatinine-adjusted to account for the differences in the urine concentration. Creatinine levels were measured in the urine using a colorimetric method (Alpha Diagnostics). The analyses were conducted according to the manufacturer's specification.

The average within the batch coefficient of variation was 8.13% and 11.57% (control urine with a low and high MT6 level, respectively) for urinary MT6s and 1.60% and 1.91% for control urine with a low and high creatinine level, respectively. Assay reliability was assessed with Pearson correlation coefficients for 76 paired measurements of morning MT6s adjusted for the creatinine levels, with $R^2 = 0.96$ ($P < 0.0001$).

Night shift work exposure assessment

The occupational data collected by means of the questionnaire included information on the current job and every previous job conducted for at least half a year. Night shift work history was analyzed using the following characteristics: the total duration of jobs with night shift work, the cumulative number of night shift hours over the entire work history, the cumulative number of night shifts, and the average frequency of night shifts weighted by the duration of the jobs. The current job characteristics were analyzed with the following variables specified: current rotating night shift work (yes/no), night shift work frequency (per month), activities conducted during the duty, duration of effective working hours during a night shift—after subtracting the time of breaks/napping from the total duration of the night shift, and light at night. Finally, the total duration of exposure to surgical lamp was calculated.

Statistical analysis

The primary goal of our analyses was to assess whether night shift work was related to mammographic density. Two measures of mammographic density were used, percentage of mammographic density and absolute density. The distribution of both of them was skewed with a long right tail. To satisfy the assumptions of the linear regression model, a square root transformation was applied to the absolute density measure. Calculated means were back-transformed. Linear regression models were used to assess the association between absolute density and night shift work. Because percentage of density expresses proportions and takes values between 0% and 100% on a continuous scale, we followed the strategy developed by Ferrari and Cribari-Neto and beta regression model was fitted (32). The goodness of fit of this model was better when compared with gauss or gamma models [Akaike information criterion (AIC); ref. 33]. Percentage of mammographic density was scaled to (0, 1) interval. Because the model requires values within the interval $0 < y < 1$, for 7 women who had percentage of mammographic density values = "0," we imputed half of the smallest value occurring in the data set (i.e., 0.00015935).

On the basis of the existing knowledge on the potential factors associated with mammographic density or melatonin synthesis, an initial list of potential confounders was formulated. Associations of these factors with percentage of mammographic density and absolute density were tested in univariate regression analyses. All the variables that passed the significance level ($P > 0.20$) were examined in the multivariate models (34). Covariates that changed the estimates of an association between absolute density or percentage of mammographic density and any of the night shift work characteristics by at least 10% were included in the final analysis, in particular: age (in years), body mass index (BMI; kg/m^2; continuous inversed values), menopausal status (premenopausal for women with periods, and postmenopausal if no menstruation occurred during the recent 12 months), age at menopause (<50, ≥50 years), age at menarche (continuous), smoking (current, past, never smokers), and the calendar season of the year when mammography was conducted (summer, autumn, winter, spring). Other characteristics were also tested, but they were not included in the final models, in particular parity (0, 1, 2, 3+), age at first birth in parous women (≤24, >24–<30, ≥ 30), family history of breast cancer (yes/no), oral contraceptives or sex hormones use (ever, never), alcohol drinking (drinks per week), physical activity [expressed in metabolic equivalents (MET) h/wk according to the standard IPAQ method], and additionally in MT6s analysis: season of the year of urine collection (March–September vs. October–February).

Analyses on cumulative exposure to night shift work were conducted in the total population and in the subgroup of women working currently night shifts. No major differences were observed and, therefore, the results for the total population are presented.

To explore the potentially modifying effect of the menopausal status or BMI, we conducted stratified analyses in premenopausal and postmenopausal women and in the categories of BMI, according to WHO classification (normal weight BMI in the range 18.5–24.99 and overweight and obese combined BMI > 24.99). The statistical significance of the effect modifier was tested using the likelihood ratio test (34). No heterogeneity of the associations was seen.

To explore the association between morning MT6s levels and mammographic percentage density, we categorized MT6s concentrations into quartiles. A linear regression model and beta regression models were fitted with absolute density and percentage density as dependent variables, respectively, and MT6s and a set of covariates described above as independent variables.

Statistical analyses were run using STATA Version 11 (StataCorp LP). All P values are 2-sided. P values less than 0.05 were considered statistically significant.

Results

The basic characteristics of the total research population and in the subgroups by the current night shift work status are presented in Table 1. The group of women currently working rotating night shifts was slightly younger, with more premenopausal women, when compared with the group of nurses working during the days only. More than 90% of the women currently working rotating night shifts had conducted such work for longer than 15 years. Among the women currently working day shifts, slightly more than 30% reported working night shifts for more than 15 years and 83% of them had resigned from night shifts more than 5 years before the starting point of our study.

The mean ± SD of percentage of mammographic density were 23.2% (±20%) and the median equaled 18.4%. The mean absolute density (±SD) was 28.1 cm^2 (±26.9 cm^2), the median: 20.7 cm^2.

Table 1. Selected characteristics of the nurses and midwives with screening mammography in the cross-sectional study

	Total population ($n = 640$)		Rotating nights ($n = 321$)		Day shifts ($n = 319$)	
Characteristics	***n* (%)**	**Mean (SD)**	***n* (%)**	**Mean (SD)**	***n* (%)**	**Mean (SD)**
Age,[a] y		49.0 (5.4)		48.1 (5.2)		50.0 (5.4)
Menopausal status						
Pre	368 (57.5)		206 (64.0)		162 (51.0)	
Post	272 (42.5)		115 (36.0)		157 (49.0)	
Age at menopause (in postmenopausal),[b] y		49.3 (3.9)		48.8 (3.9)		49.6 (4.1)
Age at menarche,[b] y		13.2 (1.5)		13.3 (1.4)		13.0 (1.6)
Number of full-term births						
0	76 (11.9)		39 (12.1)		37 (11.6)	
1	215 (33.6)		101 (31.5)		114 (35.7)	
2	313 (48.9)		156 (48.6)		157 (49.2)	
3–4	36 (5.6)		25 (7.8)		11 (3.5)	
Age at first full-term birth ($\geq$37 wk), y						
$\leq$24	319 (56.6)		159 (56.4)		160 (56.7)	
>24–<30	188 (33.3)		91 (32.3)		97 (34.4)	
$\geq$30	57 (10.1)		32 (11.3)		25 (8.9)	
Family history of breast cancer[b]						
No	587 (91.7)		292 (91.0)		295 (92.5)	
Yes	51 (8.0)		27 (8.4)		24 (7.5)	
BMI, kg/m^2		27.2 (4.7)		27.1 (4.7)		27.2 (4.7)
Smoking						
Current	188 (29.4)		106 (33.0)		82 (25.7)	
Past	173 (27.0)		77 (24.0)		96 (30.1)	
Nonsmokers	279 (43.6)		138 (43.0)		141 (44.2)	
Ever oral contraceptives or sex hormones use						
Yes	153 (23.9)		74 (23.0)		79 (24.8)	
No	487 (76.1)		247 (77.0)		240 (75.2)	
Calendar season of mammography						
Summer	135 (21.1)		63 (19.6)		72 (22.6)	
Autumn	251 (39.2)		129 (40.2)		122 (38.2)	
Winter	101 (15.8)		47 (14.6)		54 (16.9)	
Spring	153 (23.9)		82 (25.6)		71 (22.3)	
Total duration of the night shift work,[a] y						
$\leq$5	77 (12.0)		3 (0.9)		74 (23.2)	
>5–$\leq$15	163 (25.5)		26 (8.1)		137 (43.0)	
>15–$\leq$25	234 (36.6)		145 (45.2)		89 (27.9)	
>25	166 (25.9)		147 (45.8)		19 (5.9)	
Arithmetic mean − percentage of mammographic density (SD)		23.2 (20.0)		24.5 (20.4)		22.0 (19.6)
Arithmetic mean − absolute area dense, cm^2 (SD)		28.1 (26.9)		30.0 (28.4)		26.2 (25.2)

[a]$P < 0.05$ for the difference in means between the current rotating night shift nurses and non-shift nurses.

[b]Missing information for age at menopause in one person with rotating night shifts and one person on day shift; age at menarche, one night shift and two day shifts; family history was missing on 2 nurses working rotating night shifts.

Table 2. Percentage and absolute mammographic density by selected characteristics of the rotating night shift work

Night shift work characteristics	*n*	Percentage of mammographic density Adjusted[a] mean (95%CI)	Absolute density, cm^2 Adjusted[a] mean (95%CI)
Current rotating night shift			
No	319	22.5 (20.8–24.3)	21.8 (19.4–24.3)
Yes	321	23.6 (21.9–25.4)	23.9 (21.4–26.4)
P		0.62	0.25
Total duration of night shift work, y			
≤5	64	22.5 (18.8–26.1)	23.3 (17.8–28.8)
>5–≤15	165	24.5 (22.1–27.0)	23.8 (20.3–27.2)
>15–≤25	206	21.5 (19.4–23.7)	21.9 (18.8–24.9)
>25	205	23.7 (21.4–26.0)	23.1 (20.0–26.2)
P_{trend}		0.93	0.78
Total number of hours of night shift work by tertiles[b]			
I	214	24.0 (21.8–26.1)	23.8 (20.7–26.9)
II	214	22.3 (20.2–24.5)	22.5 (19.5–25.5)
III	212	23.0 (20.7–25.2)	22.3 (19.3–25.4)
P_{trend}		0.49	0.49
Cumulative number of night shifts			
≤999	185	23.8 (21.5–26.1)	23.6 (20.3–26.9)
1,000–1,999	225	22.5 (20.5–24.6)	22.9 (19.9–25.8)
2,000+	230	23.1 (20.9–25.2)	22.3 (19.4–25.2)
P_{trend}		0.66	0.56
Average frequency (weighted by duration); no. of night shifts/mo			
≤2	214	24.2 (22.0–26.3)	24.4 (21.3–27.5)
>2–≤3.9	213	22.3 (20.3–24.4)	22.8 (19.8–25.8)
>3.9	213	22.7 (20.6–24.8)	21.5 (18.6–24.4)
P_{trend}		0.33	0.18
Total duration of reported exposure to surgical lamp, y			
Day shifts (reference)	550	23.3 (21.9–24.6)	23.0 (21.1–24.9)
>0–≤10	39	21.3 (16.8–25.9)	21.1 (14.3–27.8)
>10	51	22.7 (18.4–26.9)	23.2 (17.1–29.4)
P_{trend}		0.61	0.92

[a]Adjustment for age, BMI, menopausal status, age at menopause, age at menarche, smoking, and season of the year.
[b]I tertile, ≤13,056 hours; II tertile, >13,056 to ≤24,304 hours; and III tertile, >24,304 hours.

Both percentage of mammographic density and absolute density were inversely associated with age ($P < 0.001$), the postmenopausal status ($P < 0.001$), BMI ($P < 0.001$), waist–hip ratio ($P < 0.001$), parity ($P = 0.582$), and current smoking ($P < 0.05$; data not shown).

There was no significant association between the current rotating night shift work and mammographic density (Table 2). The adjusted mean of percentage of mammographic density and absolute density were higher among the women working rotating night shifts than with those working day shifts, but the differences were not statistically significant [percentage mammographic density = 23.6% in nightshift nurses, 95% confidence interval (CI), 21.9%–25.4% vs. 22.5% in women working day shifts only, 95% CI, 20.8%–24.3%; absolute density = 23.9 cm^2, 95% CI, 21.4–26.4 cm^2 vs. 21.8 cm^2, 95% CI, 19.4–24.3 cm^2, respectively; Table 2]. Neither measure of mammographic density was statistically significantly related to the cumulative "exposure"—duration of night shift work, cumulative number of night shifts, and night shift hours during the entire work history. Surprisingly, whereas in postmenopausal women, the estimates were similar irrespective of the duration of night shift work; in premenopausal women, both percentage of mammographic density and absolute density tended to decrease with increasing duration of the exposure ($P = 0.109$ and $P = 0.069$; Supplementary Table S1).

There were no statistically significant associations between the current rotating night shift characteristics and percentage or absolute mammographic density

Table 3. Percentage of mammographic density and absolute density by selected characteristics of the rotating night shift work

Current rotating night shift work characteristics	*n*	Percentage of density Adjusted[a] mean (95%CI)	Absolute density, cm^2 Adjusted[a] mean (95%CI)
Current night shift work frequency per month			
Day shifts (reference)	319	22.5 (20.8–24.3)	21.8 (19.4–24.3)
2–7 nights	285	23.6 (21.8–25.5)	23.9 (21.2–26.5)
8+ nights	36	23.7 (18.4–29.0)	24.2 (16.7–31.7)
P		0.40	0.27
Night shifts working hours,[c] h			
Day shifts (reference)	319	22.5 (20.7–24.2)	21.8 (19.4–24.2)
<10	84	23.2 (19.9–26.5)	22.7 (18.0–27.4)
≥10–14	230	23.8 (21.7–25.9)	24.3 (21.3–27.3)
P		0.34	0.20
Light at work[c]			
Day shifts (reference)	319	22.5 (20.7–24.2)	21.8 (19.4–24.2)
Dim	139	23.0 (20.4–25.6)	23.0 (19.3–26.7)
Full light	140	24.4 (21.8–27.1)	25.1 (21.2–28.9)
Surgical lamp	35	22.8 (17.7–28.0)	22.8 (15.3–30.2)
P_{trend}		0.34	0.24
Activities at work during the night shift[c]			
Day shifts (reference)	319	22.5 (20.7–24.2)	21.8 (19.4–24.2)
1[b]	23	26.8 (19.9–33.7)	25.4 (15.9–35.0)
1 and 2 or 2	221	23.1 (21.1–25.2)	23.3 (20.4–26.3)
Any combination with 3	70	24.1 (20.4–27.8)	25.1 (19.6–30.6)
P_{trend}		0.43	0.24

[a]Adjustment for age, BMI, menopausal status, age at menopause, age at menarche, smoking, and season of the year.
[b]Categories of the activities: 1, taking care of patients, injections, administering pills; 2, small interventions; and 3, assistance in operations (deliveries).
[c]Missing information for 7 women.

(Table 3). Absolute density was 2.4 cm^2 higher in women who reported to be currently working 8 or more night shifts per month than in those only working dayshifts and 2.5 cm^2 higher in women who worked effectively more than 10 hours during night shifts than in those working dayshifts only, however, neither of these results was statistically significant. The reported exposure to light at the current job was not associated with mammographic density. We noted that in women who reported assisting in operations or deliveries at night, the mammographic density measures were higher than the reference group, but they were not statistically significantly.

None of the examined associations were modified by the menopausal status, with similar null findings both in pre- and postmenopausal women (Supplementary Tables S1 and S2)

Finally, we explored the potential association between the morning MT6s level and mammographic density, with no significant relationships found (Table 4). We also tested these associations among pre- and postmenopausal women separately, with no relationships observed in each subgroup (Supplementary Table S3). Assuming the potential for acute and reversible disturbance of the diurnal rhythm of melatonin synthesis by a night shift, we repeated the same analysis among women working during the days, only to find similar results (data not shown). We also tested the potential associations including the time between mammography and urine collection. The results did not change substantially (data not shown).

Discussion

In this study, we investigated cross-sectionally the association between rotating night shift work and mammographic density in the population of 640 nurses and midwives. We did not observe any statistically significant associations, neither when the current rotating night shift work characteristics nor when the cumulative history of night shift work were examined. We did not observe any relationships between morning MT6s levels and mammographic density, neither in the total population nor in pre- or postmenopausal women.

We found slightly higher percentage of mammographic density and absolute density in women working rotating night shifts than in day workers. The contrast to day workers was particularly observed for nurses with 8 or

Table 4. Percentage and absolute mammographic density by morning MT6s levels

	Morning MT6s[b]				
	Q1 (n = 160)	Q2 (n = 160)	Q3 (n = 160)	Q4 (n = 160)	P
Percent mammographic density [adjusted[a] mean (95%CI)]	22.6 (20.1–25.1)	23.1 (20.7–25.6)	23.2 (20.8–25.5)	23.4 (21.0–25.9)	*0.65*
Absolute density [adjusted[a] mean (95%CI)]	21.8 (18.3–25.2)	22.8 (19.3–26.2)	23.0 (19.6–26.4)	23.9 (20.3–27.4)	*0.42*

[a]Adjustment for age, BMI, menopausal status, age at menopause, age at menarche, smoking, and season of the year when mammography was conducted.
[b]Quartile ranges: Q1, ≤25.04 ng/mgCr; Q2, >25.04 to ≤42.42 ng/mgCr; Q3, >42.42 to ≤67.52 ng/mgCr; and Q4, >67.52 ng/mgCr.

more night shifts per month and nurses with night shifts of ≥10 effective working hours. However, the effects were small and not statistically significant. We did not find any significant increase in mammographic density in women who reported exposure to surgical lamps at their job or who assisted in operations or deliveries, which served as a proxy for light exposure at night.

The epidemiologic evidence on the association between night shift work and breast cancer risk has been mounting since the first report on the significant 50% increase of breast cancer risk in radio and telegraph operators in Norway was published (8). Several reviews have been conducted in the recent years (35–37) and a meta-analysis of the data conducted by Erren and colleagues (37) indicated that the risk of developing breast cancer may be increased by 70% in flight attendants and 40% in night shift workers. Increased breast cancer risk has been observed among nurses in United States (2), Norway (4, 5), Denmark (9) and other Nordic countries (38), and France (39), with the adjusted breast cancer risk up to almost 3-fold in the subgroup of Danish nurses working permanent night shifts in addition to rotating nights or day work when compared with day workers but also with a significant increase observed in rotating night nurses (OR, 1.8; ref. 9). Interestingly, the highest incidence of male breast cancer has been observed in shift or night shift work occupations in a large registry–based Nordic cohort (38). Some studies reported associations with breast cancer after long-term night shift work (≥20–30 years; refs. 2, 4, 13). In other researches, statistically significant increases have been observed after a relatively short duration of night shift work, that is, 5 to 10 years of working after midnight in Danish nurses, with OR = 2.3 (9), or more than 5 years (of ≥6 consecutive nights) with OR = 1.8 (5).

Our study, to our best knowledge, has been the first one to investigate the association between night shift work and mammographic density. The hypothesis we investigated linked occupational exposure and night shift work to mammographic density, with insight into melatonin synthesis disruption as a potential mediating factor. Melatonin receptors have been found on both ovaries and the hypothalamus, suggesting that it may interfere with the function of gonads and the gonado-hypothalamic axis (40). As shown in some experiments, it downregulates aromatase expression—an enzyme that converts androgens to estrogens (41)—and it also downregulates the transcription of the estrogen receptor (ER)α, resulting in the reduction of estrogen-related transcriptional activation (42). The data on the relationship between melatonin and sex hormones are inconsistent; nevertheless, in some studies, such relationships have been observed (43–45). The uncertainty also remains about the association between circulating estradiol and mammographic density. The associations between mammographic density and hormone-related breast cancer risk factors suggest the role of estrogens. However, the data on the link between circulating estradiol and mammographic density have been inconsistent so far (46–53).

Only one study so far has examined the links between morning MT6s and mammographic density (54) with no evidence found of an inverse association between these 2 markers. Interestingly, in the same study on Japanese women, a positive relationship between morning melatonin and mammographic density has been found in premenopausal women. The authors suggested that these findings might have been explained by the differentiation of breast cells promoted by melatonin (42). However, if melatonin is to exert the antiestrogenic action, one would have expected a decrease, not an increase in mammographic density (55–58).

The "night shift work exposure" in the population we studied was characterized by its intermittent character. The majority of the nurses working rotating night shifts in our study had been working 12-hour shifts, starting at 7 pm and ending at 7 am. This system has been operating at the hospitals in Poland since 1999. The results of our study may indicate that no risk of developing higher mammographic density is conferred by rotating night shift work or that the risk associated with such work is relatively small. We had 80% statistical power to detect a 3.5% difference in percentage of mammographic density between the groups. The increases in breast cancer risk observed among rotating night shift nurses have varied, showing a 35% increase in women having worked rotating night shifts for longer than 30 years in the Nurses

Health Study (NHS; ref. 2), up to more than 2-fold in Norwegian nurses with more than 20 years of night shift work history (4). As shown by Boyd and colleagues, a 1% difference in mammographic density is associated with a 2% increase in breast cancer risk (59). It could be thus speculated that if an increase in breast cancer risk was solely mediated by an increase in mammographic density, then the expected increase in percentage of mammographic density in nurses after 20 years of night shift work would be approximately 17% to 40%. We did not however observe any positive relationship between mammographic density and the duration of night shift work. Percentage of mammographic density in women with the duration of night shift work longer than 25 years was by 1.2% higher than in women with the night shift work duration shorter than 5 years.

The strength of this study is that it was conducted in a well-characterized population of women. As observed by other authors, nurses represent a reliable population, with usually higher participation rates and better recall than that of the general population. The response rate was relatively high with 95% mammographies conducted in the total population of women who agreed to participate in our study project. Another strength is the use of a continuous measure of mammographic density. We showed high levels of reproducibility for density readings (98%). Of the well-established associations for mammographic density, our findings confirmed inverse relationships with age, postmenopausal status, higher BMI, and waist–hip ratio. These findings support the validity of the mammography data we collected in this study.

A potential limitation of our study relates to timing of the MT6s and mammography. The average time between the urine samples collection and mammography was 120 days. We collected spot morning urine samples and determined MT6s (creatinine-adjusted), which has been recognized as a valid predictor of overnight or peak melatonin synthesis (60, 61). In our previous analysis, we had not observed a significant individual variability between the morning MT6s measurements on 2 subsequent days, based on the comparison in 23 pairs of the samples from women in the pilot phase of the study (31). Arendt has shown that the 24-hour melatonin rhythm is reproducible over a 2-week period (62). Seasonal variations of mammographic density have been suggested for premenopausal women in one study (63). If the stability of markers we analyzed over the period of several months was weak, then our analysis might have been inadequate, due to seasonal variation of both measures. In our study, we did not note a significant association between the season of the year and MT6s, and we adjusted for the season of mammography.

In summary, this study found no evidence that rotating night shift work is associated with an increase in mammographic density or that the urinary MT6s levels of melatonin are inversely associated with mammographic density. Thus, it is unlikely that the development of breast cancer in nurses working rotating night shifts observed by other researchers is mediated by an increase in mammographic density.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: B. Peplonska, E. Reszka, J.A. Lie, H. Kjuus
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B. Peplonska, A. Bukowska
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B. Peplonska, W. Sobala, W. Wasowicz, G. Ursin
Writing, review, and/or revision of the manuscript: B. Peplonska, A. Bukowska, W. Sobala, E. Reszka, W. Wasowicz, J.A. Lie, H. Kjuus, G. Ursin
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Bukowska
Study supervision: B. Peplonska, H. Kjuus
Assessed mammographic density: G. Ursin

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