ORIGINAL ARTICLE

Night shift work characteristics and 6-sulfatoxymelatonin (MT6s) in rotating night shift nurses and midwives

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ABSTRACT

Objectives Synthesis of melatonin follows a circadian cycle, with high melatonin levels during the night and low levels during the day. Light exposure at night has been hypothesised as one of potential mechanisms of breast carcinogenesis in the night shift workers through inhibition of melatonin synthesis. The aim of the study was to examine a number of determinants for night shift work in relation to 6-sulfatoxymelatonin (MT6s), primary melatonin metabolite.

Methods The cross-sectional study included 354 nurses and midwives (aged 40—60 years) currently working on rotating night shifts and 370 working days only. Data from questionnaires and 1-week diaries were used to characterise current job and total occupational history. Associations between rotating night shift work characteristics and MT6s (creatinine adjusted) in spot morning urine were tested in multiple linear regression models.

Results No significant differences were found for MT6s concentrations between women currently working on rotating night shifts and those working only day shifts (means 47.2 vs 45.7 ng/mg Cr, respectively). The adjusted means among rotating night shift nurses and midwives varied depending on the department of employment, from 35.1 ng/mg Cr in neonatology to 68.2 ng/mg Cr in the orthopaedics department. Women working eight or more night shifts per month had significantly lower MT6s levels than those having fewer night shifts per month (37.9 vs 47.4 ng/mg Cr, respectively). Total night shift work history was not associated with MT6s.

Conclusions The results of this study indicate that working eight or more night shifts per month may disrupt the synthesis of melatonin.

What this paper adds

What is already known on this subject

- Experimental studies in humans have shown that light at night transiently suppresses melatonin synthesis. Relatively few observational studies have been performed to date on melatonin metabolism and nurses' night shift work.

What this study adds

- In our study, we examined determinants of the rotating night shift work in nurses and midwives potentially influencing melatonin synthesis.

Policy implications

- The results of this study indicate that working eight or more night shifts per month may disrupt the synthesis of melatonin.

INTRODUCTION

Several epidemiological studies have found that long-term night working women have a higher risk of breast cancer than those who do not work at night.[1–5] Studies by Schernhammer *et al*[1] and Lie *et al*[3] have been conducted on rotating night shift nurses. In a recently published nested case—control study of Norwegian nurses, an increased breast cancer risk was associated with working more than five consecutive nights for more than 5 years.[6] The biological mechanisms underlying increased breast cancer risk among night shift workers have not been fully determined, although sleep deprivation, disruption of the circadian rhythm, dysregulation of the circadian rhythm genes and inhibition of the melatonin synthesis have been suggested.[7–9] An inverse association between melatonin and breast cancer risk has been observed in some but not in all prospective epidemiological studies.[10–14] In the Nurses' Health Study in the USA, the risk of breast cancer was lowered to OR of 0.62 in women with the highest melatonin synthesis (mean morning urinary 6-sulfatoxymelatonin (MT6s) ≥34.3 vs <10.2 ng/mg Cr).[14] As shown in the experimental studies, melatonin plays an important role in the regulation of immune system activity, displays strong antioxidant actions and regulates sex hormones metabolism.[15] Melatonin has been found to act as an inhibitor of tumour cell proliferation in animal models and human cancer cell lines.[16]

Synthesis and excretion of melatonin follows a circadian cycle, with peak during the night (~50 pg/ml in plasma) and low levels during the day (<10 pg/ml).[17] The rhythm is entrained by the alterations in light and darkness. Little is known on the clinical significance of changes in melatonin nocturnal synthesis. Rise in melatonin at night is associated with increased sleepiness[17] Some clinical-based research indicate potential links between melatonin and several diseases, for

example, decreased morning melatonin has been found in patients with asthma (18.%),[18] depression (23%)[19] and diabetics (40%),[20] and threefold increased nocturnal level in women with functional hypothalamic amenorrhoea[21] when compared to healthy controls.

Light exposure at night (LAN) suppresses melatonin production in mammals through an inhibition of serotonin N-acetylotransferase (AANAT).[22] Experimental studies in humans have shown that LAN transiently suppresses melatonin synthesis, with the inhibitory effects being dependent on the light brightness, that is, the brighter the light, the greater reduction in nocturnal melatonin synthesis.[23–29]

The research on the circadian rhythms under natural working conditions has focused primarily on permanent night shift workers. As has been shown, adjustment to rhythm modified by non-day work varies between workers, with phase delay, phase advance and no change in the majority of investigated permanent night workers,[30] and synchronisation occurs only until after several consecutive nights of work.[31 32] While no synchronisation to the reversed day to night rhythm could be expected after one night shift, acute effects of sleep deprivation and decrease in melatonin synthesis after LAN could affect rotating night workers. So far, only few studies have examined melatonin among rotating night nurses.[33–36] Although, their results support the association between night shift work and melatonin synthesis in the occupational group of nurses, further information on the jobs performed, and night work load or napping during the job have not been examined. The heterogeneity of the determinants of rotating night work might influence melatonin synthesis and further breast cancer risk.

To address these unanswered questions on rotating night shift work characteristics, we performed a cross-sectional study in nurses and midwives. The aim of the study was to examine a number of night shift work determinants in relation to melatonin levels, as measured by 6-sulfatoxymelatonin (MT6s). We examined questionnaire-based information on night shift work: (1) usual at current job, (2) cumulative exposure and (3) diary-based data during the 7 days before the morning urine sample collection.

METHODS

For this cross-sectional study, nurses were selected from the Local Registry of the Chamber of Nurses and Midwives in Lodz. Nurses or midwives, aged 40–60 years, currently working were eligible for the study. A total of 1117 nurses and midwives were randomly selected for the study, based on the registry database (ie, ~30% of the registered, at this age), of which 924 (83%) were contacted. Inclusion criteria were confirmed for 866 women, and 725 (84% of the eligible) women agreed to participate in the study. Morning urine samples were provided by 724 women.

A structured questionnaire was administered during in-person interviews. Data were collected on occupational history, demographics, medical and reproductive history, hormone use, physical activity (according to the International Questionnaire on Physical Activity(IPAQ)[37 38]), smoking and alcohol use, diet and sleep quality (using the Pittsburgh Sleep Quality Questionnaire, PSQI).[39] A sleep and work diary were completed by the study participants. Hours of start and end time of work were recorded for each day covering the 7 days before the urine sample collection. Measurements of weight and height were taken twice at the same occasion, and arithmetic means between the two measurements were calculated.

The study was approved by the ethical institutional review board at the Nofer Institute of Occupational Medicine. A signed informed consent was obtained from each study participant.

Night shift exposure assessment

We examined questionnaire-based information on night shift work: (1) usual at current job, (2) cumulative exposure and (3) diary-based data during the 7 days before the morning urine sample collection.

Occupational data collected via the questionnaire included information on the current job and all previous jobs held for at least 6 months, in particular, on the place of employment, the department at the hospital, the start and end dates, whether the job included night shift work, the average number of night shifts worked per week (or per month), the start and end hours of the shift, whether during these hours women napped, the total time of napping, the usual start and end time of the naps, the number of patients under her care, activities performed during the night shift and also the type of light in area where she worked. Based on the data collected, the following variables describing current night shift work were formulated: current night shift work (Yes/No), night shift work frequency (2–7, 8–14 night shifts per month), napping during the night shift (Yes/No) and napping during the hours of 23:00–3:00, total napping duration (in hours) and exposure to light at night (categories: (1) dim light, (2) full upper or side light providing the ability to read and perform activities requiring precision and (3) a surgery lamp). Hospital wards were categorised into 27 primary medical specialisations and then into four main classes, namely: (1) the surgical and intervention departments, (2) general medical care departments, (3) intensive care units, (4) diagnostics and others—not otherwise classified.

Subjects' total night shift work history was analysed using the following characteristics: total duration of jobs with night shift work (years) (calculated as the sum of the duration of each job with night shifts) and the cumulative number of night shift hours over the entire work history based on the start and end hours of the night shift for each job. Additionally, the cumulative number of night shifts was calculated using each job's duration and the average frequency of the night shifts on this job.

Based on the information collected through the 1-week diaries, we were able to assess the most recent exposure preceding the urine collection. The number of night shifts worked, the total number of night shift hours and the number of consecutive night shifts, all within the previous 7 days before the morning urine collection were calculated.

Urine samples collection and handling

We analysed 6-sulfatoxymelatonin (MT6s) in the morning spot sample to examine the effects of night shift work and in the evening spot urine to account for between-subject variability. The participants were asked to provide a spot urine sample in the evening (18:00–20:00) and the second sample next day (6:00–8:00) when the blood sample was collected and anthropometric measurements were performed. In the case of night shift workers, the urine and blood collection was synchronised with the night shift, so that the evening urine sample was collected before or soon after the night shift started, and the morning sample was taken at the end of the night shift. In the pilot phase of this study, women were asked to collect two morning samples (6:00–8:00): the same day as the evening sample and the next day. These double morning samples were collected for 45 women (20 women working on rotating night shifts and 25 on days).

The participants were asked to store the urine samples in the freezer (in 50 ml tubes) until next day collection. According to the manufacturer, storage of urine samples in the temperature 2–8°C grants stability for 4 days. The women completed the urine collection form specifying the time of urination and, in the case of the morning sample, whether this was the first void and if not, the time of the previous voids that day. Samples were collected by trained nurses and delivered to the laboratory immediately. Urine samples were frozen at −20°C in the laboratory and stored until analysis. Immediately before analysis, thawed samples were centrifuged, and the supernatant was used for MT6s determination. Urine samples were handled away from heat and sun.

Analysis of the MT6s

ELISA (DRG International Inc., New Jersey, USA) was used to determine MT6s concentration in urine. The MT6s levels were creatinine adjusted to account for differences in urine concentration. Creatinine (Cr) levels were measured in the urine using the colorimetric method (Alpha Diagnostics, Warsaw, Poland). Analyses were carried out according to the manufacturer's specifications.

The repeated MT6s and creatinine measurements in the morning and evening urine samples were performed for 77 randomly selected subjects. One outlier observation for morning MT6s was excluded. The samples were split and analysed twice, blind to the linked sample. Pearson correlation coefficients for 76 paired measurements of morning MT6s and 77 pairs of measurements evening MT6s adjusted for creatinine levels were calculated as a measure of assay reliability, with $r^2 = 0.96$ ($p<0.0001$) and $r^2 = 0.97$ ($p<0.0001$), respectively.

Statistical analysis

Arithmetic means with SDs and frequencies of the basic characteristics were calculated. The Student t test was used to compare means of the continuous variables, and the χ^2 test was calculated to compare the frequencies distribution among women working on rotating night shifts and day workers. Crude geometric means were calculated for morning MT6s concentrations.

Based on existing knowledge of factors associated with melatonin metabolism,[34 40] an initial list of the potential confounders was identified. The list included age, menopausal status (premenopausal for women who reported that they have periods and postmenopausal if reported not having periods), current smoking status, smoking within 24 h before urine collection (yes/no), season of the year of urine collection (March–September vs October–February), alcohol drinking the day before the morning urine spot collection (yes/no), tea and coffee drinking (non-drinker, infrequent drinker—up to six cups per week, and frequent drinker—drinking every day), parity (0, 1, 2, 3+), age at first birth of parous women (<20, 20 to <25, 25 to <30, 30+), age at menarche (≤16, >16 years), age at menopause, chronotype (morning vs evening type), current oral contraceptives or sex hormones use (yes/no) (ever/never), current drugs used (β-blockers, non-steroid anti-inflammatory drugs, diuretics and antidepressants combined into a group as all of them are likely to lower melatonin levels[12]), quality of sleep index (PSQI) and physical activity (expressed in metabolic equivalents MET hours per week according to the standard IPAQ method). Associations of these factors with a morning MT6s were tested in univariate regression analyses. Furthermore, tests were performed to assess the influence of the urine spot collection time (minutes since 6:00 or minutes since 18:00 for morning and evening voids, respectively), whether the sample was the first morning void (Yes/No), and the association between evening MT6s concentrations and morning MT6s levels. Evening MT6s concentrations were strongly associated with morning MT6s in the total population and both night nurses and day working nurses when analysed separately.

All variables that passed the significance level of $p<0.20$ in univariate regression were examined in the multivariate models,[41] and step forward selection method was used with 'chest' command of STATA V.11. Covariates that changed the estimate of an association between night shift work status and morning MT6s by at least 10% were included in the final regression models, in particular: age (in years); smoking (Yes/No); evening MT6s, adjusted for the time of urine collection; and age at menarche. The same approach was used for the determination of the set of covariates in the models run separately among current night shift nurses, with the following covariates retained in the final models: evening MT6s, the number of full-term births and age at menarche.

Prior research indicated that obesity is more prevalent in the night shift workers when compared to day workers.[42–46] Obesity has been also associated with melatonin synthesis.[47 48] Therefore, we considered BMI as an intermediate factor in the association between night shift work and MT6s. Analyses performed with and without BMI were compared to assess the effect of night shift work independent of BMI. No major change after inclusion of the BMI in the estimated parameters was found, and therefore, only results of the models without BMI are presented in the tables.

Due to the non-normal distribution (skewed) of morning MT6s, concentrations were transformed to the square roots of the original values (distribution of the logarithmically transformed values was not normal—$p<0.05$ Shapiro–Wilk test). Linear regression models were run with MT6s concentrations (square root transformed) as dependent variables, exposure of interest and set of covariates described above. Evening MT6s levels were log transformed.

The analyses were run in the total population and after exclusion of 10 women who moved to day work during 1 year preceding inclusion into the study. The results did not change materially in the restricted data set though and are not presented.

To explore the modifying effect of menopausal status, we performed stratified analyses in premenopausal and postmenopausal women. Statistical significance of the effect modifier was tested using the likelihood ratio test comparing appropriate likelihood statistics between models with and without interaction terms.

Based on the pilot, we analysed between-day changes and between-groups differences of morning MT6s means using mixed-effect linear regression model. Furthermore, we tested the differences between- and within-individual variability in study groups. No differences were found, and so we present the results from models assuming equal variability in both groups.

Statistical analyses were performed with STATA V.11 (StataCorp LP).

RESULTS

Selected characteristics of the nurses and midwives by their current type of work are presented in the table 1.

Rotating night nurses and midwives were slightly younger than day nurses (age difference 1.9 years), with more premenopausal women (65% vs 52%). Both the arithmetic and geometric means of the morning MT6s concentrations were similar in both groups.

A majority of night shift nurses and midwives (87.6%) reported working, on average, two to seven nights per month at the

Table 1 Selected characteristics of Polish nurses and midwives: a cross-sectional study

Characteristic	Rotating nights, (n=354)	Day shifts, (n=370)	p diff
Mean age (years), mean (SD)	48.3 (5.2)	50.2 (5.3)	0.000
Menopausal status (%)			
Premenopausal	65	52	0.000
Postmenopausal	35	48	
Age at menopause (in postmenopausal women) (years), mean (SD)	48.5 (3.9)	49.6 (4.0)	0.009
Mean age at menarche (years)†, mean (SD)	13.3 (1.4)	13.1 (1.6)	0.030
Age at first full-term birth (≥37 weeks) (years) (%)			
≤24	56	56	0.426
>24 to <30	32	35	
≥30	12	9	
Number of full-term births (%)			
0	12	13	0.040
1	31	35	
2	49	49	
3–4	8	3	
Body mass index (kg/m^2) (%)			
<25	35	38	0.504
25–30	40	40	
>30	25	22	
Smoking (%)			
Current	35	26	0.019
Past	23	31	
Non-smoker	42	43	
Tea consumption (%)			
Non-drinker	12	14	0.855
Infrequent	13	12	
Frequent	75	74	
Coffee consumption (%)			
Non-drinker	11	17	0.008
Infrequent	8	13	
Frequent	81	70	
Physical activity (MET* hours per week), mean (SD)	242 (78.4)	202 (87.9)	0.000
Chronotype† (%)			
Morning type	51	53	0.326
Evening type	49	46	
Quality of sleep PSQI (score), mean (SD)	6.4 (3.0)	6.9 (3.6)	0.002
Current oral contraceptives or sex hormones use (%)			
Yes	5	4	0.513
No	95	96	
Drugs use grouped: β-blockers, non-steroid anti-inflammatory drugs, diuretics, antidepressives (%)			
Yes	17	20	0.387
No	83	80	
Season when urine sample collected (%)			
March–September	60	50	0.008
October–February	40	50	
Alcohol consumption the day before sample collection† (%)			
Yes	3	19	0.000
No	97	81	
MT6s concentrations in morning urine (ng/mg cr)			
Arithmetic mean, mean (SD)	50.2 (1.7)	50.9 (1.9)	0.978
Geometric mean, mean (SD)	39.9 (2.2)	39.7 (2.1)	0.752
Minimum	0	3.7	
Maximum	174	242.9	

*Missing age at menarche for four women (two, rotating night shifts; two, day shift); chronotype (two, day shift); data on previous day alcohol drinking for four women (one, rotating night shift; three, day shift).

current job, with 12 h on duty, typically between 19:00 and 7:00. More than half of the women working on the rotating night shifts (53%) reported napping during the night shift, with an average nap time of 2.4 h (min: 0.25 and max: 6 h) and had been working at nights for an average of 25.4 years. Women who were currently working days only, but who had a history of night shift work, had, on average, worked 12 years on night shifts. Of these women, a majority (83%) changed to day work >5 years before their recruitment into the study. Eleven women (3%) stopped night shift work during the preceding year (data not shown).

The results of the analyses by current and total night shift (over all work) are included in table 2. No significant differences were found for MT6s concentrations between women currently working on rotating night versus those on days. Adjustments for age, smoking, evening concentration of MT6s and age at menarche slightly changed the results with MT6s of 47.2 ng/mg Cr (95% CI 44.2 to 50.2) in women working currently on a rotating night shifts and 45.7 ng/mg Cr (95% CI 42.9 to 48.6) in women working day shifts, the differences remained insignificant. Analyses involving cumulative history of night shift work did not reveal any significant relationships with MT6s levels, neither among all nurses (table 2) nor among nurses currently working rotating night shifts (data not shown).

Table 3 presents the results of analyses performed on women currently working nights. A total of 42 women reported having worked, on average, eight or more night shifts per month. In this group, the adjusted MT6s concentration was significantly lower (37.9 ng/mg Cr, 95% CI 29.8 to 44.7) when compared to women who had fewer night shifts per month (47.4 ng/mg Cr, 95% CI 44.3 to 50.6) (p difference=0.019). MT6s were insignificantly lower among women who reported assisting in operations or birth deliveries when compared to women who had no such activities on their jobs. A marginally significant association ($0.1< p<0.05$) was seen between duration of napping and MT6s levels. The MT6s level was higher in women who typically slept >2 h and took their naps between 23:00 and 3:00 (52.0 ng/mg

Table 2 Crude and adjusted means of morning MT6s concentrations by selected characteristics of rotating night shift work: a cross-sectional study of Polish nurses and midwives

Night shift work characteristic	n	Morning MT6s (ng/mg Cr), mean (95% CI)	Adjusted* morning MT6s (ng/mg Cr), mean (95% CI)
Current rotating night shift work			
No	370	45.2 (42.0 to 48.4)	45.7 (42.9 to 48.6)
Yes	354	44.9 (41.6 to 48.2)	47.2 (44.2 to 50.2)
p		0.893	0.490
Total duration of night shift work (years)			
−5	87	46.8 (40.0 to 53.6)	45.5 (39.7 to 51.3)
>5 to ≤15	182	44.6 (40.1 to 49.2)	45.9 (41.9 to 50.0)
>15 to ≤25	257	44.9 (41.0 to 48.8)	45.7 (42.1 to 49.2)
>25	198	44.8 (40.4 to 49.2)	48.4 (44.2 to 52.5)
p-trend		0.740	0.404
Total number of hours of night shift work by tertiles†			
First	242	44.5 (40.5 to 48.5)	44.0 (41.6 to 48.5)
Second	241	46.4 (42.3 to 50.4)	47.7 (44.0 to 51.3)
Third	241	44.2 (40.3 to 48.2)	46.6 (43.0 to 50.2)
p-trend		0.925	0.520
Cumulative number of night shifts			
−999 or less	210	44.7 (40.5 to 49.0)	45.2 (41.5 to 48.9)
1000–1999	253	45.9 (41.9 to 49.8)	46.6 (43.1 to 50.1)
2000+	261	44.5 (40.7 to 48.3)	47.3 (43.8 to 50.8)
p-trend		0.898	0.422

*Adjusted for age, smoking, evening MT6s and age at menarche.
†First tertile: −130 562 h, second tertile: 13 056–24 304 h; third tertile: 24 304 h.

Table 3 Crude and adjusted means of morning MT6s concentrations by selected characteristics of current rotating night shift work: a cross-sectional study of Polish nurses and midwives

Current rotating night shift work characteristic	n	Morning MT6s (ng/mg Cr), mean (95% CI)	Adjusted* morning MT6s (ng/mg Cr), mean (95% CI)
Current night shift work frequency per month (nights)			
2–7	312	45.9 (42.5 to 49.4)	47.4 (44.3 to 50.6)
8+	42	37.3 (28.7 to 45.8)	37.9 (29.8 to 44.7)
p		0.078	0.019
Activities at work during the night shift†			
1‡	27	47.6 (35.6 to 59.7)	49.4 (38.6 to 60.1)
1 and 2 or 2	241	45.8 (41.8 to 49.8)	46.1 (42.6 to 49.6)
Any combination with 3	78	41.7 (35.0 to 48.3)	45.6 (39.5 to 51.8)
p-trend		0.278	0.633
Napping during the night shift between 23:00 and 3:00†			
No	185	44.2 (39.8 to 48.6)	44.6 (40.7 to 48.5)
Yes	161	45.9 (41.1 to 50.8)	48.2 (43.8 to 52.6)
p		0.609	0.231
Duration of napping (if naps between 23:00 and 3:00) (hours)†			
0	185	44.2 (39.8 to 48.6)	44.6 (40.7 to 48.5)
≤2	72	40.5 (33.8 to 47.3)	43.7 (37.5 to 50.0)
>2	89	50.5 (43.7 to 57.3)	52.0 (46.0 to 58.3)
p		0.202	0.067
Duration of activity during the night shift (hours)†			
<10	92	50.0 (43.3 to 56.7)	51.1 (45.1 to 57.0)
≥10–14	254	43.2 (39.5 to 47.0)	44.6 (41.2 to 47.9)
p		0.079	0.060
Light at work†			
Dim	151	46.1 (41.0 to 51.1)	46.7 (42.3 to 51.1)
Full light	157	44.3 (39.4 to 49.1)	45.7 (41.3 to 50.0)
Surgical lamp	38	43.7 (34.0 to 53.5)	46.9 (38.0 to 55.8)
p-trend		0.588	0.900

*Adjusted for evening MT6s concentration, number of full-term births and age at menarche.
†Missing information for eight women.
‡Categories of the activities 1 = care for patients, injections, administering pills; 2 = small interventions; 3 = assistance in operations and births delivery.

Cr (95% CI 46.0 to 58.3) among women who slept >2 h and 44.6 ng/mg Cr (95% CI 40.7 to 48.5) among those who reported no naps). Consequently, women who reported more hours of activity (>10 h) during their night duty had a lower average MT6s level when compared to nurses who reported <10 working hours (p=0.060).

The type of light was not associated with morning MT6s concentrations. There were differences in the reported LAN exposure by hospital departments, with high prevalence of exposure to surgical lamp reported by nurses working at the operating suite (70%) and birth delivery room (87%), gynaecology and obstetrics (7%) and none in some other department, including orthopaedics. In the subgroup of 21 women who reported some work under a surgical lamp and did not take naps during their shift, the adjusted morning MT6s mean was 41.6 ng/mg Cr (95% CI 30.3 to 52.8). The corresponding mean among the 17 women who napped was 53.7 ng/mg Cr (95% CI 39.7 to 68.3); this difference was statistically insignificant (data not shown).

No modification of the associations by menopausal status was found (data for premenopausal and postmenopausal women are shown in the supplemental tables 1–3). Inverse association between night shift frequency, duration (hours) of work at night with adjusted morning MT6s was statistically significant in premenopausal women only.

No statistically significant relationships between the night shift work load during the week preceding the morning MT6s determination and MT6s concentrations were identified (table 4). However, the mean MT6s concentrations tended to be lower with increasing numbers of night shift hours, with a 17% difference in the MT6s means between the small group of women who had two or more consecutive nights when compared to nurses with one night shift only (38.5 ng/mg Cr (95% CI 25.8 to 51.2) vs 46.5 ng/mg Cr (95% CI 43.5 to 49.5)).

The analysis for departments did not reveal significant differences (table 5). The lowest adjusted morning MT6s mean was found among women employed in the neonatology

Table 4 Mean MT6s concentrations by type of night shift work during the 7 days preceding the morning urine sample collection: a cross-sectional study of Polish nurses and midwives

Characteristic (No. of women)	Morning MT6s (ng/mg Cr), mean (95% CI)	Adjusted* morning MT6s (ng/mg Cr), mean (95% CI)
Night shifts during preceding week		
1 (88)	47.9 (41.2 to 54.5)	46.8 (41.0 to 52.7)
2 (209)	43.3 (39.2 to 47.5)	46.2 (42.4 to 50.0)
>3 (57)	46.0 (37.8 to 54.2)	44.9 (37.7 to 52.1)
p	0.587	0.695
Night shifts hours during preceding week		
≤24 (273)	44.7 (41.0 to 48.3)	46.7 (43.3 to 50.0)
>24 (81)	45.5 (38.7 to 52.4)	44.5 (38.5 to 50.4)
p	0.830	0.528
Consecutive nights immediately before morning urine collection		
1 (339)	45.2 (41.9 to 48.5)	46.5 (43.5 to 49.5)
≥2 (15)	38.1 (23.6 to 52.6)	38.5 (25.8 to 51.2)
p	0.367	0.252

*Adjusted for age, evening MT6s concentration, number of full-term births and age at menarche.

Table 5 MT6s concentrations among night shift nurses by hospital department (medical specialty): a cross-sectional study of Polish nurses and midwives

Category of the departments (No. of women)	Morning MT6s (ng/mg Cr), mean (95% CI)	Adjusted* morning MT6s (ng/mg Cr), mean (95% CI)
Surgical and intervention department (93)	42.4 (36.2 to 48.5)	45.0 (39.4 to 50.5)
Intensive care units (63)	42.1 (34.7 to 49.5)	43.4 (36.8 to 50.0)
General care dep. (155)	49.1 (44.0 to 54.2)	49.5 (45.1 to 54.4)
Other, including diagnostics (43)	39.9 (31.2 to 48.7)	40.7 (32.9 to 48.5)
Hospital department		
Gynaecology and obstetrics (27)	36.1 (25.7 to 46.4)	41.8 (32.1 to 51.6)
Intern (37)	49.4 (39.0 to 59.8)	47.5 (38.7 to 56.4)
Neonatology (13)	34.8 (20.1 to 49.4)	35.1 (22.4 to 47.8)
Surgery (22)	46.1 (33.1 to 59.1)	46.4 (35.1 to 57.7)
Otolaryngology (12)	53.2 (34.3 to 77.1)	59.7 (36.9 to 70.5)
Operating suite (20)	44.1 (30.8 to 57.4)	45.1 (33.4 to 56.8)
Orthopaedics (11)	67.2 (45.0 to 89.3)	68.2 (48.7 to 87.7)
Intensive care (60)	41.1 (33.7 to 48.6)	42.5 (35.9 to 49.0)
Infectious diseases ward (17)	59.1 (42.4 to 75.8)	58.4 (44.0 to 72.8)
Admission room (26)	50.2 (37.7 to 62.6)	53.4 (42.1 to 64.7)
Birth delivery room (16)	42.7 (28.0 to 57.3)	44.8 (31.7 to 57.9)
Other then hospital (41)	41.4 (32.4 to 50.4)	42.0 (34.1 to 49.9)

*Adjusted for evening MT6s concentration, number of full-term births and age at menarche.

department (35.1 ng/mg Cr, 95% CI 22.4 to 47.8), and the highest mean was for women in the orthopaedics department (68.2 ng/mg Cr, 95% CI 48.7 to 87.7) (table 5). When nurses employed in the intensive care unit (the largest subgroup) were used as the reference, the only difference that reached statistical significance was recorded for women working in the infectious diseases and the orthopaedics wards. These results did not changed when frequency of the night shift and nap time were introduced into the model (data not shown).

Based on the data collected during the pilot phase of this study, it was possible to compare morning MT6s concentrations for night shift nurses before and after the night shift, controlling for time of morning urination. No significant difference of morning MT6s levels was found between the two measurements (45.9 ng/mg Cr, 95% CI 27.8 to 64.1 before the shift and 39.1 ng/mg Cr, 95% CI 23.4 to 54.9 after the shift, p=0.784) (data not shown). The corresponding values for nurses working on days were 46.3 ng/mg Cr (95% CI 31.0 to 61.6) and 43.9 ng/mg Cr (95% CI 28.5 to 59.2) on the day 1 and day 2, respectively. The difference between groups on the day 1 was insignificant (p=0.578) as well as difference in mean levels changes between days (p=0.797).

DISCUSSION

The results of this cross-sectional study showed no difference in the average morning MT6s concentration of current rotating night shift nurses and midwives and of women working only days. Neither were any significant differences observed when morning MT6s means were compared in a small pilot group before and after the night shift. Furthermore, we did not see associations between MT6s and cumulative measures of night shift work.

Subgroup analyses restricted to women currently employed on the rotating night shifts suggest that specific characteristics of night work, such as the high frequency of the night duties and long working night hours, might influence MT6s levels. In particular, eight or more night shifts per month were associated with a significant decrease in MT6s concentrations. Activity of >10 h while working showed a borderline significant association with MT6s.

Relatively few observational studies have been performed to date on melatonin metabolism and nurses' night shift work. Analysis of 170 nurses by Hansen *et al* revealed a statistically significant ~20% difference in the MT6s concentrations estimated for the early morning urine samples among night shift nurses when comparing their working and non-working days.[33] Schernhammer *et al*[35] in a small study of 80 premenopausal nurses reported that the average melatonin concentration fell by 67% among nurses who had more than four night shifts when compared to women who had no night shifts during the two preceding weeks. Another study of Schernhammer *et al*[34] of 459 women (including 80 from the previous analysis) showed MT6s levels 26% lower among women with more than four night shifts over the previous 2 weeks, but this difference was no longer statistically significant. The results of our study are consistent with this report, although the magnitude of the reduction in melatonin levels in the present study were only ~20% among women who reported to work, on average, eight or more night shifts per month when compared to those who worked fewer night shifts. The information based on the interview served as a proxy of average exposure. Analysis based on the diaries allowed us to examine data on the night shifts during 1 week preceding sample collection. No statistically significant associations were found. It is worth noting, however, that the MT6s means were lower by ~17% for a small group of women (n=15) who reported having worked two or more consecutive nights.

Our study suggests that potential consequences of night shift work may depend on the department worked. About half of the women who currently work on night shifts reported napping, including naps in the window of 23:00–3:00. In Poland, regulations on night shift work do not specify whether napping is or not allowed. Similar habits have been reported by nurses in a study in France, with typical naps at night reported by nine of 20 nurses.[49] In our study, MT6s concentrations were higher (although insignificantly) in nurses who napped longer when compared to those who did not nap, suggesting that napping may counteract the effects of night work or maybe lowering the potential 'exposure'. Analysis by hospital department found some between-group variation in the MT6s means. We found the lowest after shift MT6s means among nurses working in neonatology. The mean was half of the highest mean found among nurses employed in the orthopaedics department. Among women employed in the neonatology department, 69% reported no napping in the night, while 46% of nurses in orthopaedics did not nap. As well, the mean time for the napping differed, with 0.7 h in the neonatology and 1.4 h in orthopaedics. However, the estimates remained unchanged when these additional terms were introduced into the models, indicating that other factors may be affecting the observed differences. Further studies on larger populations are warranted to confirm our findings.

In our study, no significant associations between reported average light exposure on duty and the mean MT6s were observed. Only 38 reported 'exposure' to a surgical lamp in addition to the two other light categories, and we did not find any effect of this exposure. However, we were not able to characterise exposure to light during the night preceding urine collection and in particular the timing and duration of the light

exposure during that night. We admit that using a proxy measure for light exposure (at average night duty) may have introduced some misclassification of recent night light exposure.

In the earlier experiments simulating night work over room light (~150 lx), no effects on body temperature, plasma cortisol, urine production, alertness and cognitive performance were observed in a study by Czeisler *et al*, while these parameters have been synchronised to night work schedule with 1-week long light exposure to 7000 lx during night hours.[50] In a more recent study of young volunteers, it has been reported that even small changes in light exposure (~100 lx) in the evening hours may significantly affect plasma melatonin concentration and human circadian pacemaker[51] In Poland, regulations specify the minimum light intensity at hospitals at night as follow: at the intensive therapy room—at the nurses desk 20 lx, 3 lx in hospital corridors, 100 lx at the patient's bed, 500 lx in operating suites and 5000–50 000 lx at the operating table. As far as we know, data on LAN in Polish hospitals are not available. In some other countries, light intensity at hospitals at night was reported to be around 150 lx.[49 52] In the intensive care unit in Italy, the background light intensity was ~20 lx in the study by Costa *et al*.[53] In the same study, no suppression of melatonin was observed after bright light exposure (2350 lx) for two consecutive nights at workplace. Personal light exposure assessments with individual metres may add to verification whether exposure to bright light at night in hospitals, including an evaluation of intensity, timing and duration of the light exposure, is important for blocking melatonin synthesis.

In our study, we determined MT6s, a primary metabolite of melatonin in spot morning urine sample, which has been recognised as a valid predictor of overnight or peak melatonin synthesis[54 55] and has been used in other epidemiological studies.[14 34–36] Future studies analysing MT6s in samples of the urine in every void over night or analysing MT6s concentration in the total urine collected during night hours might clarify further the circadian disruption pattern in this occupational group.

In summary, we found no difference in the morning MT6s levels between rotating night shifts nurses and midwives and those working days only. However, our results indicate that working more than eight night shifts per month, and probably two or more consecutive nights, might disrupt melatonin synthesis. Further field studies are warranted to clarify the role of LAN on the synthesis of melatonin.

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