Final Progress Report

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List of Terms and Abbreviations

CRC: Colorectal cancer

BMI: Body Mass Index

NHS2: Nurses' Health Study 2

OR: Odds Ratio

RR: Relative Risk

95% CI: 95% Confidence Interval

Abstract (498 words)

Rotating Night Shifts, Sleep Duration, and the Risk of Colorectal Adenoma
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Night-shift work has been associated with cancer risk and is considered a “probable carcinogen”. Occupation exposure to light at night, can lead to disrupted levels of melatonin, a hormone intimately involved in the body’s internal clock and that has been shown decrease cancer cell growth in animal and laboratory studies. Disrupted melatonin levels likely increase the risk of developing cancer.

Colorectal cancer (CRC) is the third most common cancer in the United States. CRC often starts first as an adenoma (polyp). Adenoma that are detected/removed will no longer develop into cancer. Night shift work has been associated with CRC, but few have evaluated whether night-shift work contribute to the early stages of CRC (e.g., adenoma).

Studies suggest that habitual duration of sleep increases risk of death from cancer, cardiovascular disease, and other causes. However, only one prior study has evaluated sleep duration and colorectal cancer.

We evaluated whether working rotating night shifts, habitual sleep, and directly measured melatonin levels were associated with colorectal adenoma. We used data from the Nurses’ Health Study II (NHS II), a large cohort of over 116,000 women who have been followed biennially since 1989 for lifestyle, diet, medication use, and health outcomes.

Using self-reported sleep duration and history of night-shift work, we analyzed whether longer duration of night shift-work or habitual sleep duration were associated with colorectal adenoma. We did not find any evidence of an association. We ran secondary analyses by sub-site within the colon, and also by the type of adenoma (low risk versus high risk), but did not observe any statistically significant variation in risk. We combined the null results from Aim 1 and Aim 3 into one manuscript that was published in 2017: “Rotating night shift work, sleep, and colorectal adenoma in women”.

We evaluated whether lower melatonin levels were associated with risk of distal colorectal. We assayed stored urine samples for melatonin (aMT6s) in a subset of our study population. Using a nested case/control design, we evaluated whether the risk of distal, high-risk colorectal adenoma (most likely to develop into cancer) varied by levels of aMT6s. We did not find any evidence of an association. We ran secondary analyses by subgroups of smoking and body mass index (BMI), and results were equivocal and non-statistically significant. We have prepared a manuscript of these results that will be submitted for publication.

Although we found no evidence for an association between night shift work, sleep duration, urinary melatonin, and the risk of colorectal adenoma, we cannot rule out the possibility that these associations exist. Our study was limited by sample size, particularly for sub-group analyses. Next steps from this project include pooling our data with other data sources, accruing additional follow-up from this study, and/or evaluating other adenoma outcomes (multiple, serrated, etc.). We have generated valuable, novel data from the melatonin assays for future studies. Exposure to light at night and sleep remain important modifiable occupational risk factors for cancer; many opportunities still exist to intervene to improve worker safety in this area.

Section 1

Significant or Key Findings

Using self-reported sleep duration and history of night-shift work in the setting of a large, established, prospective epidemiologic cohort study, we analyzed whether longer duration of night shift-work or habitual sleep duration were associated with colorectal adenoma. We did not find any evidence of an association. We ran secondary analyses by sub-site within the colon, and also by the type of adenoma (low risk versus high risk), but did not observe any statistically significant variation in risk. We combined the null results from Aim 1 and Aim 3 into one manuscript that was published in 2017: "Rotating night shift work, sleep, and colorectal adenoma in women".

Using data from a subset of the large cohort, we evaluated whether lower melatonin levels were associated with risk of distal colorectal. We assayed stored urine samples for melatonin (aMT6s) in a subset of our study population. Using a nested case/control design, we evaluated whether the risk of distal, high-risk colorectal adenoma (most likely to develop into cancer) varied by levels of aMT6s. We did not find any evidence of an association. We also ran secondary analyses by subgroups of smoking and body mass index (BMI), and results were equivocal and non-statistically significant.

Translation of Findings

Although we found no evidence for an association between night shift work, sleep duration, urinary melatonin, and the risk of colorectal adenoma, we cannot rule out the possibility that these associations exist. Based on our existing understanding and current literature, night-shift work, exposure to light at night, and sleep remain important modifiable occupational risk factors for cancer.

If we were able to demonstrate evidence that these risk factors contribute to the early stages of carcinogenesis, this would translate to possibly implementing personalized screening recommendations (starting at an earlier age or more frequent screening) for individuals who have a history of night shift work or high overnight light exposure. In addition, if urinary melatonin was associated with increased risk, testing for melatonin levels could potentially be used in the clinical setting for risk stratification of individuals who should receive specific and targeted counseling regarding screening, as well as possibly a different risk screening regimen.

Research Outcomes/Impact

Our findings will not have an immediate impact on occupational safety and health because overall our results were null. However, this novel study is still one of the first to evaluate the hypothesis that night-shift work and sleep influence risk of colorectal adenoma in the setting of such a large, well-defined study with the ability to adjust for multiple confounders. To our knowledge, this is also the first study to use direct measures of an individuals' melatonin levels using stored urine samples, which is superior to other studies that have collected 24-hour urine rather than first morning urine. We have proven the ability to use stored urine to measure melatonin as an occupational exposure. Our publications (one pending) contribute to a currently sparse literature in this area, and will stimulate additional studies in other populations that can help confirm our findings. Publishing these findings will also possibly lead to possible collaborations in the future that will have more statistical power and that will lead to clearer results.

Section 2

Scientific Report

Aim 1 and Aim 3

1) **Longer duration of night shift-work is associated with an increased risk of colorectal adenoma.**
2) **Longer (and shorter) habitual duration of sleep are associated with a higher risk of colorectal adenoma.**

ROTATING NIGHT SHIFT WORK, SLEEP, AND COLORECTAL ADENOMA IN WOMEN

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ABSTRACT

Purpose: To investigate the associations of rotating night shift work history and sleep duration with risk of colorectal adenoma.

Methods: We evaluated 56,275 cancer-free participants of the Nurses' Health Study II, who had their first colonoscopy or sigmoidoscopy between 1991 and 2011; rotating night shift work and sleep duration were reported by mailed questionnaire. Multivariable-adjusted logistic regression was used to estimate relative risks (RR) of colorectal adenoma, with 95% confidence intervals (CI), across categories of rotating night shift work history (none, 1-4, 5-9, and ≥10 years) and sleep duration (≤5, 6, 7, 8, and ≥9 hours/day).

Results: We found no association between duration of rotating night shift work and occurrence of colorectal adenoma (p-trend across shift work categories=0.5). Women with the longest durations of rotating night shift work (≥10 years) had a similar risk of adenoma compared to women without a history of rotating night shift work (multivariable-adjusted RR: 0.96, 95% CI: 0.83-1.11). Similarly, there were no associations of shorter or longer sleep durations with adenoma risk (p-trend=0.2 across sleep durations of ≤5 through 7 hours/day, and p-trend=0.5 across sleep durations of 7 through ≥9 hours/day). Results were similar when we examined associations according to adenoma location and subtype.

Conclusions: Our results do not support an association between rotating night shift work or sleep duration and risk of colorectal adenoma in women.

KEYWORDS

Rotating night shift work, sleep, colonoscopy, polyps, adenoma, women

INTRODUCTION

The World Health Organization classified shift work as a probable carcinogen, largely based on studies of breast cancer[1]. However, evidence for colorectal cancer is increasing, and a recent meta-analysis suggested that longer durations of shift work might also be associated with a higher risk of colon cancer [2]. Sleep disturbances may mediate this association or act independently to increase risk of this outcome[3]. An important precursor for most colorectal cancers is adenomatous polyps (i.e., adenoma), making them an appealing target for interventions [4,5]. Yet, to date, only one hospital-based case-control study has examined night shift work and sleep characteristics in relation to colorectal adenoma [6]. We hypothesized that longer duration of rotating night shift work and extreme sleep durations might be associated with greater risk of adenoma in the Nurses' Health Study II (NHS II).

METHODS

Study population

The NHS II began in 1989, when 116,430 female registered nurses, aged 25-42 years and living in the United States, completed a mailed questionnaire about lifestyle factors and medical history. Similar questionnaires updated this information biennially; response rates have been ≥90% for every questionnaire cycle. Information on shift work history was collected at baseline and repeatedly throughout follow up, and sleep duration was assessed once in 2001. Women began reporting lower endoscopy procedures and findings of colorectal polyps in 1991, which was considered "baseline" for our analyses.

We identified 56,275 women without a diagnosis of cancer (excluding non-melanoma skin cancer), inflammatory bowel disease, ulcerative colitis, familial polyposis, or colorectal polyps by 1991, who reported first-time lower gastrointestinal endoscopy (colonoscopy or sigmoidoscopy) between 1991 and 2011. We excluded women without an initial report of shift work history (n=286) and information on sleep duration (n=7,044), leaving 55,989 women for analyses of shift work history and adenoma risk and 49,231 women for analyses of sleep duration and adenoma risk. The Institutional Review Boards of the Brigham and Women's Hospital and the Harvard T. H. Chan School of Public Health approved this study.

Ascertainment of colorectal adenoma

Women who reported a new diagnosis of colorectal polyps on cohort questionnaires were asked for permission to obtain their medical records. Study investigators, who were blinded to participants' exposure status, reviewed medical records and pathology reports to confirm adenoma cases; they also extracted information on anatomic location, size, number, and histological type of adenoma. For our analyses, we considered adenoma cases that were diagnosed on first lower endoscopy and confirmed by pathology report.

Ascertainment of shift work history and sleep duration

Women reported their total duration of rotating night shift work at baseline in 1989 (never, 1-2, 3-5, 6-9, 10-14, 15-19, and ≥20 years), and updated this information on biennial questionnaires in 1991, 1993, 1997, 2001, 2005, and 2007 (for each two-year period: none, 1-4, 5-9, 10-14, 15-19, and ≥20 months). Because the 1995, 1999, and 2003 questionnaires did not include this question, retrospective assessments of rotating night shift work were included on the 2001 and 2005 questionnaires. We assigned participants the value of the midpoint of their response category, and summed these values across all questionnaires through the year of first lower endoscopy.

Women reported their usual sleep duration in a 24-hour period on the 2001 questionnaire; response categories were: <5, 5, 6, 7, 8, 9, 10, and ≥11 hours.

Statistical analysis

Age- and multivariable- adjusted logistic regression models were used to estimate odds ratios (OR), approximating relative risks (RR), and 95% confidence intervals (CI) for overall colorectal adenoma and adenoma by location and subtype across categories of rotating night shift work history (none, 1-4, 5-9, and ≥10 years) and sleep duration (≤5, 6, 7, 8, and ≥9 hours/day). Non-cases were women without adenoma (or with hyperplastic polyps only) detected at first lower endoscopy; women with no history of rotating night shift work and sleep durations of 7 hours/day comprised the reference categories, respectively. Linear trends were evaluated using the midpoint of each rotating night shift work category, and separately for sleep durations of ≤5, 6, and 7 hours/day and sleep durations of 7, 8, and ≥9 hours/day. We utilized a Bonferroni correction to determine the threshold at which p-values would be considered significant; therefore, we divided the p-value of 0.05 by 33 (the number of comparisons for which a p-value was calculated in our main analyses) and considered $p<0.002$ as indicating statistical significance.

In secondary analyses, we evaluated associations among women who underwent lower endoscopy for screening purposes only, and restricted to adenoma cases occurring after women reported sleep duration in 2001. We also evaluated effect modification by body-mass index and joint effects of rotating night shift work and sleep duration by adding interaction terms to our models.

RESULTS

After adjusting for potential confounding factors, we found no association between rotating night shift work history and overall risk of colorectal adenoma (p-trend=0.5), and similar adenoma risks for women across all categories of rotating night shift work, compared to women with no history of rotating night shift work (e.g., RR: 0.96, 95% CI: 0.83-1.11 comparing extremes of ≥10 years of rotating night shift work history versus none) (Table 1). Rotating night shift work was also unrelated to risks of proximal, distal, and rectal adenoma (p-trends were 0.9, 0.7, and 0.3, respectively), as well as large (≥1 cm), small (<1 cm), advanced (large, villous, or high-grade dysplasia), non-advanced (small and tubular), multiple (≥2 polyps), high-risk (advanced or ≥3 polyps), and low-risk (non-advanced and 1-2 polyps) adenoma subtypes.

In addition, there was no overall association between shorter or longer sleep durations and adenoma risk in multivariable models (p-trend=0.2 across sleep durations of ≤5 through 7 hours/day, and p-trend=0.5 across sleep durations of 7 through ≥9 hours/day) (Table 1). We did observe a suggestion of a decreased adenoma risk for women with sleep durations ≤5 hours/day compared to 7 hours/day (multivariable-adjusted RR: 0.83, 95% CI: 0.69-1.01), whereas the adenoma risk was comparable for women with sleep durations ≥9 hours/day versus 7 hours/day (multivariable-adjusted RR: 0.91, 95% CI: 0.76-1.09). When Bonferroni correction was applied, no significant trends of shorter or longer sleep durations emerged by adenoma location or subtype.

In secondary analyses, results were similar when we restricted our sample to women who underwent lower endoscopy for screening purposes only, and to cases occurring after women reported sleep duration in 2001. There was no effect modification by body-mass index and no joint effect of rotating night shift work and sleep duration on adenoma risk.

DISCUSSION

We identified no overall association of rotating night shift work history or sleep duration with risk of colorectal adenoma in women. Similarly, these exposures were not related to adenoma risk when considering different anatomic locations or subtypes separately. Thus, our results do not support the hypothesis that longer duration of rotating night shift work or extremes of sleep duration increase the risk of colorectal adenoma.

Our findings are consistent with results from a previous epidemiologic study of night shift work and colorectal adenoma. In that study, there was no difference in the prevalence of adenomas comparing participants with a history of night shift work to those without such a history (OR: 1.16, 95% CI: 0.85-1.59)[6], although information on duration of night shift work was not available. Thus, our study extends these results by suggesting that no association exists even among participants with up to ten years of shift work history. Given the growing evidence that shift work may be associated with an increased risk of colorectal cancer[2], inconsistent results for colorectal adenoma versus cancer (as occurred in the Nurses' Health Studies) might suggest that circadian disruption acts more as a cancer promoter than initiator.

Few observational studies have examined associations between sleep duration and risk of colorectal cancer[9-11], and the only study that previously examined this association found a 50% increased risk in colorectal adenoma with shorter sleep duration (OR 1.49, 95% CI: 1.02-2.19)[6]. However, the authors did not account for confounding by important lifestyle factors (e.g., diet and physical activity) in their analyses; therefore, this result could be explained at least in part by residual confounding.

Limitations of our study should be noted. First, rotating night shift work and sleep duration were assessed by questionnaire, which likely resulted in non-differential exposure misclassification. Multiple studies have identified associations of shift work history and sleep duration with chronic disease in this cohort, but such misclassification could still have contributed to our null findings. Second, our study was conducted among women only, and results may not apply to men.

In conclusion, our study does not provide evidence supporting the hypothesis that longer history of rotating night shift work or extremes of sleep duration increase the risk of colorectal adenoma in women.

COMPLIANCE WITH ETHICAL STANDARDS

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Table 1. Relative risks of colorectal adenoma, overall and by location and subtype, across categories of rotating night shift work history and sleep duration in the Nurses' Health Study II

	Rotating night shift work history (in years)				
	None	1-4	5-9	≥10	p-trend [a]
Overall adenoma [b]					
Number of cases	936	1,425	409	244	
Multivariable-adjusted RR (95% CI) [c]	1.00 (reference)	0.93 (0.85, 1.01)	0.98 (0.87, 1.11)	0.96 (0.83, 1.11)	0.5
Proximal colon					
Number of cases	427	653	210	115	
Multivariable-adjusted RR (95% CI) [c]	1.00 (reference)	0.93 (0.82, 1.05)	1.08 (0.91, 1.28)	0.95 (0.77, 1.18)	0.9
Distal colon					
Number of cases	430	680	196	122	
Multivariable-adjusted RR (95% CI) [c]	1.00 (reference)	0.96 (0.85, 1.08)	1.02 (0.86, 1.21)	1.04 (0.85, 1.28)	0.7
Rectum					
Number of cases	177	241	65	43	
Multivariable-adjusted RR (95% CI) [c]	1.00 (reference)	0.83 (0.69, 1.01)	0.85 (0.64, 1.13)	0.93 (0.66, 1.30)	0.3
Large (≥1 cm)					
Number of cases	220	316	105	60	
Multivariable-adjusted RR (95% CI) [c]	1.00 (reference)	0.87 (0.73, 1.04)	1.07 (0.85, 1.36)	1.00 (0.74, 1.33)	0.8
Small (<1 cm)					
Number of cases	670	1,039	282	169	
Multivariable-adjusted RR (95% CI) [c]	1.00 (reference)	0.94 (0.85, 1.04)	0.94 (0.82, 1.09)	0.92 (0.77, 1.10)	0.3
Advanced (large, villous histology, or high-grade dysplasia)					
Number of cases	267	396	122	74	
Multivariable-adjusted RR (95% CI) [c]	1.00 (reference)	0.90 (0.77, 1.06)	1.03 (0.83, 1.28)	1.01 (0.77, 1.31)	0.9
Non-advanced (small and tubular)					
Number of cases	496	751	211	116	
Multivariable-adjusted RR (95% CI) [c]	1.00 (reference)	0.91 (0.81, 1.02)	0.94 (0.80, 1.11)	0.85 (0.69, 1.05)	0.1
Multiple (≥2 polyps)					
Number of cases	180	292	111	57	

Multivariable-adjusted RR (95% CI) [c]	1.00 (reference)	0.97 (0.80, 1.16)	1.32 (1.04, 1.68)	1.09 (0.80, 1.47)	0.1		
High risk (advanced or ≥3 polyps)							
Number of cases	297	438	140	82			
Multivariable-adjusted RR (95% CI) [c]	1.00 (reference)	0.90 (0.77, 1.05)	1.06 (0.86, 1.30)	1.01 (0.78, 1.29)	0.7		
Low risk (non-advanced and 1-2 polyps)							
Number of cases	476	722	194	113			
Multivariable-adjusted RR (95% CI) [c]	1.00 (reference)	0.91 (0.81, 1.03)	0.90 (0.76, 1.07)	0.86 (0.70, 1.07)	0.1		
	Sleep duration (in hours/day)						
	p-trend [a]	≤5	6	7	8	≥9	p-trend [a]
Overall adenoma [b]							
Number of cases	--	124	672	1,242	672	141	--
Multivariable-adjusted RR (95% CI) [c]	0.2	0.83 (0.69, 1.01)	1.00 (0.90, 1.10)	1.00 (reference)	1.00 (0.90, 1.10)	0.91 (0.76, 1.09)	0.5
Proximal colon							
Number of cases	--	59	313	599	294	56	--
Multivariable-adjusted RR (95% CI) [c]	0.2	0.83 (0.63, 1.10)	0.96 (0.83, 1.10)	1.00 (reference)	0.91 (0.79, 1.05)	0.76 (0.58, 1.01)	0.03
Distal colon							
Number of cases	--	58	330	576	319	63	--
Multivariable-adjusted RR (95% CI) [c]	0.4	0.81 (0.61, 1.06)	1.04 (0.91, 1.20)	1.00 (reference)	1.02 (0.89, 1.17)	0.86 (0.66, 1.12)	0.6
Rectum							
Number of cases	--	20	110	211	123	32	--
Multivariable-adjusted RR (95% CI) [c]	0.3	0.78 (0.49, 1.24)	0.96 (0.76, 1.21)	1.00 (reference)	1.07 (0.86, 1.34)	1.20 (0.83, 1.75)	0.3
Large (≥1 cm)							
Number of cases	--	31	166	264	157	39	--
Multivariable-adjusted RR (95% CI) [c]	0.8	0.92 (0.63, 1.34)	1.13 (0.93, 1.38)	1.00 (reference)	1.10 (0.90, 1.34)	1.14 (0.81, 1.60)	0.3
Small (<1 cm)							
Number of cases	--	84	481	909	479	95	--
Multivariable-adjusted RR (95% CI) [c]	0.1	0.79 (0.63, 0.99)	0.98 (0.88, 1.10)	1.00 (reference)	0.97 (0.87, 1.09)	0.85 (0.69, 1.06)	0.2
Advanced (large, villous histology, or high-grade dysplasia)							
Number of cases	--	41	198	330	186	50	--

Multivariable-adjusted RR (95% CI) [c]	0.8	0.98 (0.71, 1.37)	1.08 (0.90, 1.29)	1.00 (reference)	1.04 (0.87, 1.25)	1.18 (0.87, 1.59)	0.3
Non-advanced (small and tubular)							
Number of cases	--	56	356	670	345	69	--
Multivariable-adjusted RR (95% CI) [c]	0.09	0.72 (0.55, 0.96)	0.99 (0.87, 1.13)	1.00 (reference)	0.94 (0.83, 1.08)	0.84 (0.65, 1.09)	0.2
Multiple (≥2 polyps)							
Number of cases	--	26	142	262	126	36	--
Multivariable-adjusted RR (95% CI) [c]	0.3	0.77 (0.51, 1.15)	0.97 (0.79, 1.19)	1.00 (reference)	0.89 (0.72, 1.11)	1.07 (0.75, 1.53)	0.7
High risk (advanced or ≥3 polyps)							
Number of cases	--	44	219	365	216	56	--
Multivariable-adjusted RR (95% CI) [c]	0.8	0.96 (0.70, 1.32)	1.08 (0.91, 1.28)	1.00 (reference)	1.09 (0.92, 1.30)	1.19 (0.90, 1.59)	0.1
Low risk (non-advanced and 1-2 polyps)							
Number of cases	--	54	340	647	326	63	--
Multivariable-adjusted RR (95% CI) [c]	0.08	0.72 (0.54, 0.96)	0.98 (0.86, 1.12)	1.00 (reference)	0.92 (0.81, 1.06)	0.80 (0.61, 1.04)	0.07

CI=confidence interval; RR=relative risk

[a] Due to multiple comparisons, a Bonferroni-corrected threshold of $p<0.002$ was considered statistically significant.

[b] Adenoma found in the proximal and/or distal colon, and/or in the rectum.

[c] Models are adjusted for age, time-period of first lower endoscopy, reason for endoscopy, family history of colorectal cancer, height, body-mass index, physical activity, pack-years of smoking, alcohol intake, menopausal status, menopausal hormone use, oral contraceptive use, multivitamin use, total calcium intake, supplemental vitamin D intake, red meat intake, aspirin use, non-steroidal anti-inflammatory drug use, and predicted vitamin D score.

Aim 2

1) **Lower melatonin secretion (assessed as urinary melatonin [aMT6-s] levels) is associated with an increased risk of distal colorectal adenoma and this association will be particularly strong for large/advanced adenoma, which have higher malignant potential.**

URINARY MELATONIN CONCENTRATIONS AND RISK OF COLORECTAL ADENOMA IN THE NURSES' HEALTH STUDY 2

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Abstract

Background: Sleep duration and shift work have been associated with risk of colorectal cancer. However, data on the association between melatonin, which is decreased among people exposed to light at night, and the risk of colorectal cancer is limited. Furthermore, understanding whether melatonin contributes to the risk of the precursor lesion of colorectal cancer will allow insights into what stage of carcinogenesis at which melatonin disruptions may be acting. This study aims to evaluate the association between urinary melatonin levels and the risk of incident advanced colorectal adenoma in a long-standing cohort study of women.

Methods**:** In a case-control study of colorectal cancer nested within the Nurses' Health Study 2, we measured 6-sulfatoxy melatonin (aMT6s) in urine samples collected between 1997-1999 and followed participants until 2011, resulting in 397 cases and 397 matched controls. aMT6s concentrations were corrected for creatinine levels, a measure of urine concentration. We used conditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the association of aMT6s with risk of advanced colorectal adenoma, adjusting for known colorectal adenoma risk factors.

Results: Urinary aMT6s levels were not associated with risk of colorectal adenoma. Compared to women in the bottom quartile, women in the top quartile had essentially the same risk of colorectal cancer (OR: 0.997, 0.66 – 1.50 95% CI). Results did not vary by smoking status at urine collection (ever vs. never), or body mass index at urine collection (<25 vs. ≥25), though there was a suggestion of a higher risk among women with a BMI 25 or greater in the highest quartile of melatonin.

Conclusions: We found no compelling evidence supporting an association between urinary melatonin levels and incident development of advanced colorectal adenoma, though our analyses were limited by sample size, particularly for the stratified analyses. Additional studies in larger populations or that utilize pooled data is needed to further clarify the potential association between aMT6s and the risk of colorectal adenoma.

Background

Colorectal cancer is the third most common cancer in men and women (1). Colorectal cancer is a unique neoplasm because there is a known pre-clinical benign lesion, e.g. adenoma, which can be detected and removed, thereby fundamentally altering the natural history of the disease. This underscores how imperative it is to evaluate the association between a risk factor and colorectal adenoma if we hope to fully understand its role in the pathogenesis of colorectal neoplasia.

The central circadian pacemaker (suprachiasmatic nuclei of the hypothalamus, or SCN) controls the vast majority of circadian rhythms, including that of pineal melatonin biosynthesis.(8) Melatonin (5-methoxytryptamine) is an indoleamine produced primarily by the pineal gland that is secreted exclusively during the dark phase of the light-dark cycle (9). The pineal gland releases peak levels of the hormone in the middle of the night (~ 2 a.m.), with daytime melatonin concentrations that remain relatively low (10). Melatonin is thus considered the internal marker of day and night, and is acutely sensitive to environmental light exposure. Levels of melatonin's major urinary metabolite, 6-sulfatoxymelatonin (aMT6s), as measured in the first morning urine like in our cohort, are very closely correlated with plasma melatonin levels in blood and saliva (11-18). However, in contrast to single plasma or saliva melatonin measures, a single measure of aMT6s in morning urine allows determination of nightly peak levels of melatonin, which is what we are interested in. Because most other studies collected 24-hour urine rather than first morning urine, we are one of the few prospective cohorts who have first morning urine to evaluate melatonin (19).

Melatonin has direct effects on organ systems including scavenging of free radicals as well as receptor mediated effects (via its two receptors, MT_1 and MT_2) (20). Moreover, reports show that melatonin is oncostatic in a variety of tumor cells: *in vitro* studies indicate that both pharmacological and physiologic doses of melatonin reduce the growth of malignant cells of the breast (21-25) and other tumor sites (26-30). Most mechanisms proposed to explain the oncostatic action of melatonin include the hormone's antimitotic and antioxidant activity (31) as well as potential modulation of cell-cycle length through effects on the p53-p21 pathway (24). In sum, experimental evidence for a tumor-protective effect of melatonin is strongest for breast tumors to date, but has also been demonstrated in a variety of other tumor cell lines.

Several studies confirm that night shift workers tend to have lower melatonin levels than those who work day shifts due to their exposure to light at night. One study identified a significant reduction of melatonin after approximately 2 weeks of intermittent night light exposure (32). An analysis of aMT6s in Danish nurses also found that those women working night shifts had a significantly lower urinary concentration than those working the day shift ($p<0.01$) (33). Similarly, another study confirmed that night shift nurses had lower levels of aMT6S during night work compared with either their days off or day shift work (34).

Because of the strong relationship between shift work and melatonin levels, we propose to evaluate whether urinary melatonin levels are associated with the risk of colorectal adenoma directly. We will exclude shift workers from these analyses, because their physiologic melatonin peak will likely not have occurred at the time of our urine collection (which took place in the morning). However, if melatonin levels are inversely associated with colorectal adenoma risk in individuals whose circadian rhythm is not disrupted by inappropriate light exposure as in night work, this would imply that the suppression of melatonin that occurs during night work may be an important factor on the causal pathway to colorectal adenoma, and ultimately colorectal cancer. Hence, our results will help clarify whether melatonin is part of the biological mechanism

explaining the effect of shift work. Alternately, melatonin could be an independent predictor of risk due its multiple biologic actions.

Methods

In 1989, 116,434 female registered nurses in the US between ages 25 and 42 years, residing in 14 of the larger states, completed an initial questionnaire on risk factors for cancer and cardiovascular disease, forming the Nurses' Health Study 2 cohort. The population is predominantly Caucasian, reflecting the ethnic background of women entering nursing in the US in the 1970's and 80's. Since 1989, the cohort has been followed by mailed questionnaires sent every two years to update exposure information and to ascertain non-fatal incident diseases.

Identification of Cases

On each biennial questionnaire, we asked whether participants had undergone sigmoidoscopy or colonoscopy; what the indications for these procedures were; whether colon or rectal polyps had been diagnosed; and, if they had, the date of diagnosis. When a participant reported a diagnosis, we obtained her informed consent to acquire medical records and pathology reports. Study investigators who were blinded to exposure data reviewed all records and extracted data on the histologic type, anatomic location, and size of polyps. We did not ask participants to specify whether sigmoidoscopy or colonoscopy was performed. On the basis of secular trends, there was probably a gradual increase in the relative proportion of colonoscopies compared with sigmoidoscopies. Because we assumed that a substantial portion of all procedures were sigmoidoscopies, which encompass only examination of the distal colon and rectum, we included only adenomas of the distal colon (descending and sigmoid colon) and rectum to prevent misclassification and potential detection bias. Women with adenomas proximal to the descending colon, but without synchronous distal adenoma, were included as non-case participants. We defined case subjects as those with one or more pathology-verified adenomas less than 60 cm from the anus.

Case and control selection.

We selected one control for each case, matched on age, month of specimen collection, menopausal status, fasting status, time of day of blood collection, and luteal day of menstrual cycle. Based on previous case-control studies nested within the NHS2 cohort (plasma sex steroids and prolactin), controls were matched to cases on a number of additional matching criteria, not all of which are relevant for the current grant proposal (e.g., fasting status). We will exclude women who reported current night shift work from the pool of cases and controls eligible for selection. For the laboratory analyses, case and control pairs were processed and assayed together, but blinded and in scrambled order.

Urine Collection (1997-1999) in the NHS2 cohort

Women who had not previously reported a diagnosis of cancer and were responders to the most recent NHS2 study questionnaire were eligible for the sample collection. We sent a letter to each eligible participant to invite her participation. Nurses were asked to provide first morning urine samples between 1996 and 1999. In total, 29,616 women participated, and collection kits were sent to them. Along with the urine collection kit, we sent a sub-questionnaire asking whether, and if so, how many night shifts they had worked in the two weeks before urine collection. Urine samples were processed immediately after their return to our laboratory and archived in liquid nitrogen freezers. 19,092 of the samples were timed within the menstrual cycle

of a woman (i.e., timed samples), and 10,521 were collected at random time points (i.e., random samples). For women with timed samples, a urine sample was collected in the luteal phase only. Although the freezer nitrogen levels are automatically monitored, we also checked them manually each week.

Quality assurance/Validity of urinary melatonin measurements.

Both morning urinary melatonin (aMT6s) and creatinine-corrected aMT6s account for approximately 70% of the total plasma melatonin measured from the previous night (18). Various studies have demonstrated that measurements of aMT6s in urine and plasma correlate highly with each other and have found the correlation to be between 0.80 and 0.94 (12, 15-18) In particular, peak nocturnal plasma melatonin has been significantly related (r=0.94) to morning aMT6s (16). These results have recently been confirmed in older women (16), in whom neither menopausal status nor hormonal replacement therapy altered melatonin measurements in morning void urine. We assessed laboratory reproducibility and the validity of our collection and processing methods (54) in the NHS2 cohort. We found an intraclass correlation for two samples collected 2.8 years apart from 80 women for creatinine-adjusted aMT6s of 0.72 (95 % CI, 0.65, 0.82).

Laboratory Assay

aMT6s measurements were assayed using commercially available ELISA kits (ALPCO, Windham, NH). The Bühlmann 6-SMT ELISA is an immunoassay using an antibody-capture technique with a lower detection limit of 0.8 ng/ml for aMT6s. All aMT6s assays will be creatinine-standardized to account for differences arising from variations in urine concentrations. The CVs for urinary aMT6s were <14% and for creatinine <9% (55, 56). Creatinine levels are also measured for each sample by the same laboratory, using creatinine reagents purchased from Sigma Diagnostics, and aMT6s levels are normalized to the creatinine level of the sample to account for differences arising from variations in urine concentrations.
We matched one control per case on year of birth, date of endoscopy, month of blood collection, and fasting status. Matched cases and controls were handled identically and together, shipped in the same batch, and assayed in the same analytical run. The order within each case-control pair was random. We have previously worked with this laboratory, which is run by an expert in the field of interest, Dr. Vincent Ricchiutti. We have monitored precision by routinely adding approximately 5% of repeated quality control samples.
We used conditional logistic regression to account for the matching structure of the data. We modeled urinary aMT6a as a continuous variable, as well as dividing it into quartiles (based on the distribution in the controls) and used the median of each quartile to test for trend. We generated models that were age and matching factor adjusted, as well as multivariate models that included possible confounding variables.

Results

Cases and controls were generally similar, though controls had slightly higher urinary aMT6-s levels, had slightly lower BMIs, were less likely to be a smoker, were less likely to have worked night shifts, ate less meat and drank less alcohol (Table 1). Overall, urinary aMT6s levels were not associated with risk of advanced distal colorectal adenoma. Compared to women in the bottom quartile, women in the top quartile had essentially the same risk of colorectal cancer (OR: 0.997, 0.66 – 1.50 95% CI). After additional adjustment for potential confounding factors (model 2), the results were essentially unchanged (Table 2). Further stratified analyses results did not support any effect modification by smoking status at time of urine collection (ever vs.

never), or by body mass index at urine collection (<25 vs. ≥25; Table 3). A suggestion of higher risk among women the women with BMI 25 or greater, particularly in the highest quartile of melatonin, was intriguing, but difficult to interpret given the wide confidence intervals.

Discussion

The evidence supporting an association between night shift work, sleep patterns, and the risk of colorectal cancer is growing. Despite this, few have studied the association between these circadian-related factors and the risk of colorectal adenoma. Even fewer studies have direct measures of melatonin levels as a marker for circadian disruption. We evaluated the association between urinary melatonin and the risk of colorectal adenoma in a well-established and long-running cohort study of women. The data allowed us to control for various confounding factors, and we had a reasonably long follow-up time to observe colorectal adenoma incidence. We were limited to adenoma in the distal portion of the colorectum and to “high risk” adenoma, though these are the lesions most likely to progress to frank carcinoma. If we had observed an association, it would provide evidence that circadian disruption, due to night shift work for example, influences peak nighttime melatonin levels and thereby causing a biological effect on the development of adenoma.

We know that some risk factors for colorectal cancer act either earlier or later in the process of normal colorectal mucosa developing towards adenocarcinoma. Our results do not support an association between a one-time measure of melatonin levels, measured during first morning void and the risk of distal advanced adenoma, but we were limited by relatively small sample sizes. It is possible that melatonin could have a differential effect on colorectal adenoma that arise in the proximal colon, as we know some risk factors vary according to anatomic location; future studies that can include those cases should be undertaken. Melatonin may also act depending on the specific molecular pathology of the adenoma and eventual carcinoma – more granular data on type of adenoma or multiple adenoma may provide further insight into the observed effects of melatonin on colorectal cancer.

Table 1. Age-standardized characteristics of the study population at baseline (1999) by adenoma case control status NHS II.

	Case-control status	
	Cases (n=397)	Controls (n=397)
Age at urine collection[a]	45.59 ± 4.35	45.53 ± 4.32
Urinary aMT6-s (ng/mg creatinine)	51.63 ± 34.83	56.48 ± 80.17
Family history of colon or rectal cancer, %	8	7
Height(inches)	64.67 ± 2.81	64.72 ± 2.67
BMI (kg/m2)	26.13 ± 5.51	25.66 ± 5.36
Physical activity (MET-hrs/week)	15.80 ± 18.39	16.89 ± 17.84
Smoking (pack-years)	11.40 ± 78.60	10.12 ± 79.53
Smoking status		
- Never smoker, %	87	94
- Past smoker, %	10	4
- Current smoker, %	3	2
History of night shift work		
- Never, %	42	39
- 1-5 years, %	45	46
- 6+ years, %	13	15
Usual duration of sleep (hours)[b]		
- =<5 hrs sleep, %	5	4
- 6 hrs sleep, %	21	22
- 7 hrs sleep, %	42	45
- 8 hrs sleep, %	26	24
- >=9 hrs sleep, %	5	5
Total folate intake (mcg/day)	592.8 ± 250.8	632.2 ± 270.6
Red and processed meat intake in 1999 (g/day)	0.2541 ± 0.1899	0.2108 ± 0.1885
Alcohol intake (gm/day)	3.572 ± 6.146	2.984 ± 5.686
Multivitamin use in 1999, %	58	58
Aspirin use, %	17	18
Post-menopausal hormone (PMH) use in past 6 months		
- Yes, %	26	30
- No, %	20	15
- Premenopausal/missing, %	54	55

Values are means ± SD or percentages and are standardized to the age distribution of the study population.
Values of polytomous variables may not sum to 100% due to rounding

[a] Matching factor

[b] Reported in 2001

[*] Value is not age-adjusted

Table 2. Odds Ratios for Advanced Distal Colorectal Adenoma by Quartile of Urinary 6-Sulfatoxymelatonin Concentration (aMT6a) in the Nurses' Health Study II.

Quartile of aMT6a	Median (ng/mg creatinine)	No of Cases	No of Controls	Model 1[a]		Model 2[b]	
				OR	95% CI	OR	95% CI
1	16.8	108	100	1.00 (ref)		1.00 (ref)	
2	37.4	92	99	0.864	0.58 – 1.29	0.821	0.54 – 1.25
3	56.6	91	99	0.858	0.58 – 1.27	0.896	0.59 – 1.36
4	84.4	106	99	0.997	0.66 – 1.50	1.018	0.66 – 1.58
P for trend					0.81		0.96

[a.] Matching factors only

[b.] Matching factors and height (inches), physical activity (MET-hrs/wk), BMI (kg/m^2), history of shift work (never, 1-5 yrs, 6+ years), sleep duration (≤5, 6, 7, 8, ≥9 hours), alcohol intake (g/day), multivitamin use (yes/no), aspirin use (yes/no), family history of colon or rectal cancer (yes/no), calcium intake (g/day), PMH use in last 6 months (yes/no), red and processed meat intake (g/day), and smoking (pack-years).

Table 3. Odds Ratios for Advanced Distal Colorectal Adenoma by Quartile of Urinary 6-Sulfatoxymelatonin Concentration (aMT6a) in the Nurses' Health Study II by smoking status and BMI.

Quartile of aMT6a (ng/mg creatinine)	No of Cases	No of Controls	Model 1[a] OR	95% CI
Non-Smokers				
1	64	72	1.00 (ref)	
2	57	73	0.77	0.40 – 1.47
3	59	70	0.94	0.49 – 1.82
4	75	68	1.31	0.66 – 2.61
P for trend				0.64
Ever Smokers[b]				
1	44	28	1.00 (ref)	
2	35	26	0.39	0.01 – 12.1
3	32	29	0.43	0.02 – 10.8
4	31	31	0.05	0.00 – 7.82
P for trend				0.23
BMI <25				
1	59	48	1.00 (ref)	
2	51	47	0.97	0.32 -3.00
3	46	43	0.98	0.36 – 2.66
4	50	39	0.92	0.32 – 2.66
P for trend				0.90
BMI 25+				
1	44	28	1.00 (ref)	
2	35	26	1.16	0.39 – 3.48
3	32	29	1.04	0.35 – 3.11
4	31	31	2.45	0.79 - 7.62
P for trend				0.20

[a.] Matching factors and height (inches), physical activity (MET-hrs/wk), BMI (kg/m^2), history of shift work (never, 1-5 yrs, 6+ years), sleep duration (≤5, 6, 7, 8, ≥9 hours), alcohol intake (g/day), multivitamin use (yes/no), aspirin use (yes/no), family history of colon or rectal cancer (yes/no), calcium intake (g/day), PMH use in last 6 months (yes/no), red and processed meat intake (g/day), and smoking (pack-years).
[b.] Current and past smokers as reported in 1999.

Publications

1. Rotating night shift work, sleep, and colorectal adenoma in women. Devore EE, Massa J, Papantoniou K, Schernhammer ES, Wu K, Zhang X, Willett WC, Fuchs CS, Chan AT, Ogino S, Giovannucci E, Wei EK. Int J Colorectal Dis. 2017 Jul;32(7):1013-1018. doi: 10.1007/s00384-017-2758-z. Epub 2017 Jan 17. PMID: 28097381
2. Urinary melatonin concentrations and risk of colorectal adenoma in the Nurses' Health Study 2. Esther K Wei, Elizabeth Poole, Kana Wu, Edward Giovannucci, Shuji Ogino, Walter C. Willett, Charles S. Fuchs, Eva Schernhammer. Pending.