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A Nested Case-Controlled Comparison of Telomere Length and Psychological Functioning in Breast Cancer Survivors with and without Insomnia Symptoms

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Abstract

The ability to achieve sufficient restorative sleep is important in the maintenance of physical and mental health; however, disturbed sleep and insomnia symptoms are a common experience among women with breast cancer. In non-cancer populations, insufficient sleep quantity and quality has been associated with shortened telomere length (TL), a measure of accumulated cellular damage and human aging. This feasibility study compared TL in women previously diagnosed with breast cancer with clinically significant insomnia symptoms ($n=70$) to an age- and body mass index (BMI)-matched comparison group ($n=70$) of breast cancer survivors. Women with significant insomnia symptoms had higher levels of unemployment compared to women without insomnia. TL was positively skewed and shorter in the insomnia group (*Median* = 6.000, $S=1.000$, standard error [SE] = 0.287) than the control group (*Median* = 6.195, $S=-0.269$, SE = 0.287); however, this was not significant ($p=0.29$). Women with insomnia also reported significantly higher levels of depression ($p<0.001$), anxiety ($p<0.001$), and fatigue ($p<0.001$). This study provides the first measure of effect size and variability of TL in women with breast cancer and highlights the need for larger sample sizes to investigate the impact of insomnia and co-morbid symptom distress on cellular aging.

Introduction

Depending on cancer stage and/or phase of treatment, estimates suggest that 20%–70% of women with breast cancer report difficulty with disturbed sleep and insomnia symptoms.[1] Although the precise factors have yet to be fully elucidated, previous research indicates that undergoing chemotherapy, the hormonal changes associated with treatment, and elevated levels of psychological distress and fatigue in women with breast cancer place this group at an increased risk for insomnia symptoms and dysregulated sleep/wake rhythms.[2]

The ability to achieve sufficient restorative sleep is important in the maintenance of physical and psychological well-being. Telomere length (TL) is increasingly being examined as a biomarker of accumulated cellular damage and human aging. Telomeres are repetitive nucleoprotein structures on the ends of chromosomes that protect against genome instability and unbridled cellular division. With each cell division, TL progressively shortens until critically short telomeres eventually lead to cell cycle arrest or cell death. Telomere shortening occurs via normal aging but can be accelerated through exposure to oxidative stress. In the only study to date examining the association between sleep and oxidative stress, Gulec et al.[3] demonstrated that patients with primary insomnia have significantly higher levels of oxidative stress indicators and lower levels of free radical scavenging anti-oxidants. This suggests a potential link between sleep, oxidative stress, and TL as a pathway to health or disease.

Emerging research supports the relationship of poor sleep quality/insufficient sleep quantity and shorter TL. Prather

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et al.[4] examined associations between sleep duration and quality with leukocyte TL in a sample of 245 healthy midlife women. In individuals with disturbed sleep for greater than 3 months, the association between sleep quality and TL was significant, even after adjusting for perceived psychological stress. Similarly, a significant linear association between longer telomeres and increased sleep duration has been shown for men, adjusting for age, body mass index (BMI), depression, and other relevant co-variates.[5] Using population-based data from the Nurses' Health Study, Liang and colleagues [6] investigated associations between sleep duration and TL in 4117 women. A curvilinear relationship between sleep duration and shorter TL was reported for women sleeping less than 6 hr and greater than 10 hr, after adjusting for age, BMI, and smoking status. Although these studies are informative in the general area of sleep and health, very little is known about sleep and biological outcomes in individuals with cancer, who, by virtue of their disease and treatment, are at a higher risk for disrupted sleep and potential negative health consequences.

To lay the foundation for future research, we performed a nested case–control study of post-menopausal women with breast cancer to examine the variability of TL and provide effect size estimates in women with and without clinically significant insomnia symptoms.

Methods

Sample and setting

Post-menopausal women with non-metastatic primary breast cancer were drawn from a cross-sectional study conducted between March, 2008, and July, 2009, at the Rowan Breast Cancer Center of the Abramson Cancer Center of the University of Pennsylvania (Philadelphia, PA). Research assistants screened medical records and approached potential patients for enrollment at their regular follow-up appointments. After informed consent was obtained, each participant completed a self-administered survey, and a blood sample was collected for telomere analysis. The institutional review boards of the University of Pennsylvania and the Abramson Cancer Center approved the study and all participants provided informed consent. Patients were eligible for study inclusion if they were age 18 or older; had a history of stage I, II, or III breast cancer; were post-menopausal; and could understand written English. Women with metastatic breast cancer were excluded because of the potential impact of disease and continued active treatment(s) on cellular function. Demographic and cancer history information was confirmed or abstracted through chart review.

The sample size in the original cross-sectional survey was 476, reflecting a 78% response rate among those eligible. Women were selected for the insomnia group if their Insomnia Severity Index (ISI) score was in the moderate (15–21 points) or severe (22–28 points) range. The ISI is a seven-item measure of subjective insomnia symptoms in the past 2 weeks and has been validated for use in cancer patients.[7] Considering the association between age, BMI, and TL,[8] each case was matched with a control from the remaining participants who did not report significant insomnia symptoms (ISI score < 7), and were similar in age (within ± 2 years) and BMI (± 2 units) using the 'psmatching' program through the SPSS R-Plugin. The final sample included 70 women with moderate to severe insomnia symptoms and a matched comparison group of 70 women without insomnia symptoms ($n = 140$).

Measures

Primary outcome. Mean terminal restriction fragment (TRF) lengths were determined as described by Lorenzini et al.[9] with minor modifications. Purified DNA (500 ng) isolated from peripheral blood mononuclear cells (PBMCs) was digested to completion with HinfI and RsaI. Digested samples and size markers (^{32}P-end-labeled a 1 kb + DNA ladder and HindIII-cut lambda DNA) were separated in a 0.5% agarose gel. Under denaturing alkaline conditions, the blots were in-gel hybridized with a ^{32}P-end-labeled oligonucleotide $(CCCTAA)_4$ probe overnight at 55°C. Blots were washed to remove non-specifically bound probe, and visualized using a PhosPhorImager (Molecular Dynamics). Mean TRF length was calculated as $\Sigma(OD_i)/\Sigma(OD_i/L_i)$, where OD_i is the total radioactivity above background in interval i and L_i is the average length of i in base pairs (bp). Undigested DNA from each of the samples that were used in the TRF assay underwent low-resolution agarose gel electrophoresis and stained with ethidium bromide to verify that all DNA was of high-molecular-weight material as the presence of low-molecular-weight material would indicate a degradation of the telomere DNA, resulting in an underestimation of TL by the TRF assay. All DNA was verified to be of high molecular weight, indicating accurate TLs were obtained by TRF.

Secondary outcome. The Hospital Anxiety and Depression Scale (HADS) is a 14-item, self-rated instrument for anxiety (seven items) and depression (seven items) symptoms in the past week and has been extensively used in people with cancer.[10] Established cutoffs are: 0–7 not significant, 8–10 subclinical, and 11–21 clinically significant depression/anxiety.

The Brief Fatigue Inventory (BFI) is a nine-item self-report questionnaire assessing the presence of fatigue in the past week with three items assessing present, usual, and worst severity of fatigue in the past 24 hr and six specific items concerning the degree to which fatigue interferes with activity, mood, walking, work, relationships, and enjoyment of life.[11] A BFI total score of more than three points is an indicator of clinically significant fatigue.

Analysis

The Shapiro–Wilk test was used to determine whether the continuous data was normally distributed. Paired samples *t*-tests, Wilcoxon-signed rank tests, or McNemar chi-squared analyses were used to compare demographic, cancer history, and clinical variables as appropriate for the matched pairs. Wilcoxon signed rank tests for were used to compare TL and levels of fatigue, depression, and anxiety in women with insomnia symptoms to the age- and BMI-matched comparison group. Effect size (ES) was calculated using the Cohen's *d* for each outcome measure using the original standard deviations and adjusting for the correlation between groups. Spearman rank-order correlations were conducted to assess the association between TL, fatigue, anxiety, and depression in the insomnia group. All analyses were two sided, with 0.05 indicating significance.

Results

In the entire sample ($n = 140$), there was an overall effect of age on TL (coefficient $\beta = -14.98$, 95% confidence interval [CI] -27.14 to -2.83, $p = 0.016$). Demographic and clinical characteristics of the insomnia and matched comparison groups are provided in Table 1. Women with breast cancer and clinically significant insomnia symptoms did not differ from the comparison group in terms of age, BMI, time since diagnosis, race, education, marital status, cancer stage, chemotherapy, number of co-morbidities, or anti-depressant use. There was a significant difference, however, between the two groups in regard to employment status, with the insomnia group reporting higher levels of unemployment ($p < 0.001$) and a trend for the women with insomnia to report a lower education level ($p = 0.06$). These variables were not significantly associated with TL and were therefore not considered to be confounders.

Table 1. Demographic and Clinical Characteristics

	Insomnia group n = *70*	*Matched comparison* n = *70*	p[a]
Demographic characteristics			
Age			
Mean (SD)	59.87 (9.57)	59.83 (9.40)	
Range	42–84	41–84	0.98
BMI			
Mean (SD)	27.92 (5.76)	28.09 (6.09)	
Range	19.3–44.7	19.4–45.7	0.88
Race			
White	57	52	
Non-white[b]	13	18	0.41
Education			
High school	16	10	
College	37	30	
Graduate	17	30	0.06
Employment			
Full time	26	40	
Part time	12	10	
Unemployed	32	20	0.001
Marital status			
Married/partnered	45	41	
Single	22	28	0.61
Clinical characteristics			
Time since diagnosis			
Mean days (SD)	1783.14 (1744.49)	1917.37 (1790.22)	0.66
Cancer stage			
0&I	30	26	
II	29	36	
III	11	8	0.67
Chemotherapy			
None	28	21	
Yes w/o taxanes	19	21	
Yes w/ taxanes	23	28	0.25
Anti-depressant use			
Yes	19	12	
No	51	58	0.25
Co-morbidity			
None	16	13	
One	19	18	
Two or more	35	39	0.70

The paired samples *t*-test or Wilcoxon-signed rank test was used as appropriate for continuous variables.

[a]McNemar test was used for variables with two categories; McNemar–Bowker was used for variables with more than two categories;

[b]Mostly black.

Despite significantly higher levels of fatigue ($p < 0.001$, ES = 1.32), anxiety ($p < 0.001$, ES = 0.79), and depression symptoms ($p < 0.001$, ES = 0.80), the TL of women with more severe insomnia symptoms was not significantly different from the matched comparison group ($p = 0.29$, ES = 0.09) (Table 2). However, the distribution of TL in the women with more severe insomnia symptoms was significantly positively skewed (*Median* = 6.000, $S = 1.000$, SE = 0.287) when compared to the women without sleep difficulty (*Median* = 6.195, $S = -0.269$, SE = 0.287). TL was not significantly associated with insomnia severity ($p = 0.31$), fatigue ($p = 0.31$), anxiety ($p = 0.59$), or depression ($p = 0.67$) in the insomnia group.

Discussion

This study is the first to explore the relationship of insomnia severity and TL in post-menopausal women with breast cancer. Women with more severe insomnia symptoms reported significantly higher levels of depression, anxiety, and fatigue; however, in contrast to studies published with non-cancer populations, TL did not differ by insomnia severity. In the context of this feasibility study, a possible lack of association may mean: (1) There was no true association, or (2) the study may be under-powered for detecting the difference. We investigated this possibility by conducting a *post hoc* sample size analysis for matched pairs using the observed telomere means, standard deviation, an effect size of 0.09, and an alpha of 0.05. Using these figures, a total sample size of 994 patients would be required to achieve 80% power. Considering that the risk of making a Type II error (*i.e.*, not rejecting the null hypothesis when in fact the alternate hypothesis is true) was high, it would be premature to make conclusions about the relationship of insomnia and TL. As such, this study provides a foundation for future research and highlights the need to recruit and accommodate larger sample sizes.

Theoretically, poor sleep quality/quantity may produce cellular damage and negatively influence TL by increasing levels of inflammation and oxidative stress.[3] Research has demonstrated an association between sleep/wake disruption and flattened diurnal cortisol rhythms in women awaiting breast cancer surgery.[12] Furthermore, in breast cancer patients undergoing chemotherapy, poor sleep quality has been associated with increases in inflammatory cytokines (interleukin-6 and interleukin-1 receptor agonist) and C-reactive protein.[13] In samples of healthy individuals, disrupted sleep quality/quantity have been demonstrated to have cascading neuroendocrine effects, including increased inflammatory cytokine production[14] and reduced immune function[15]; however, the relationship of poor sleep to cancer processes and outcomes is only beginning to be understood. Additionally, a high level of subjective stress has been associated with both insomnia and shorter TLs.[16,17] Stress reduction interventions have been shown to improve insomnia in individuals with cancer.[18] There is also preliminary research suggesting that a stress reduction intervention may positively

Table 2. Case–Control Analysis of Telomere Length, Fatigue, Anxiety, and Depression in Women with Breast Cancer

Variable	*Insomnia group* n = *70*	*Matched comparison* n = *70*	p	*ES*
Telomere length				
Mean base pairs (SD)	6112.29 (637.67)	6203.00 (753.59)	0.29	0.09
BFI fatigue				
Mean (SD)	4.35 (2.20)	1.20 (1.47)	<0.001	1.32
HADS anxiety				
Mean (SD)	8.00 (3.75)	4.17 (3.34)	<0.001	0.79
HADS Depression				
Mean (SD)	5.54 (2.02)	3.19 (1.91)	<0.001	0.80

ES, effect size; SD, standard deviation; BFI, Brief Fatigue Inventory; HADS, Hospital Anxiety and Depression Scale.

impact TL in a sample of cervical cancer survivors.[19] This suggests that the modification of stress appraisals may be one mechanism to improve health at a cellular level. Although the exact relevance of these changes to functioning and cancer recovery is unknown, they are suggestive of important, and possibly modifiable, biobehavioral pathways to improve outcomes.

Considering that this is a preliminary examination of TL and insomnia, the following limitations require consideration. First, a case–control design seeks to identify an association rather than infer causation. Future prospective research needs to define the causal relationship between insomnia and TL. Insomnia was assessed via self-report; however, the ISI has been widely used in cancer populations and has established sensitivity and specificity to detect cases.[7] Second, future research may want to include ambulatory monitoring of sleep via actigraphy or ambulatory polysomnography for an objective means of testing the association between sleep and TL. Despite matching for BMI, we are unable to rule out the presence of sleep apnea, which is associated with higher oxidative stress and poorer overall sleep quality. Future studies should ensure that the sample is as homogeneous as possible by thoroughly screening for other psychiatric and sleep disorders to provide the strongest test possible. Future studies may want to include measures of telomerase activity, inflammatory cytokines, and oxidative stress markers to better understand the underlying mechanisms that may link sleep to health outcomes and even possibly cancer survival.

In line with previous studies, women with greater insomnia severity reported significantly more depression, anxiety, and fatigue. The cumulative effect of this symptom burden can reduce overall quality of life and possibly accelerate cellular aging. Considering the prevalence and potential burden of sleep disturbances and insomnia if left untreated, their early identification and treatment may have the potential to prevent and/or mitigate the psychological and physical consequences of cancer.

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Author Disclosure Statement

No competing financial interests exist.

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