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Religiousness, Spirituality, and Salivary Cortisol in Breast Cancer Survivorship: A Pilot Study

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Abstract

Background—Psychoneuroimmunological theory suggests a physiological relationship exists between stress, psychosocial-behavioral factors, and neuroendocrine-immune outcomes; however, evidence has been limited.

Objective—The primary aim of this pilot study was to determine feasibility and acceptability of a salivary cortisol self-collection protocol with a mail-back option for breast cancer survivors. A secondary aim was to examine relationships between religiousness/spirituality (R/S), perceptions of health, and diurnal salivary cortisol (DSC) as a proxy measure for neuroendocrine activity.

Methods—This was an observational, cross-sectional study. Participants completed measures of R/S, perceptions of health, demographics, and DSC.

Results—The sample was composed of female breast cancer survivors (N= 41). Self-collection of DSC using a mail-back option was feasible; validity of mailed salivary cortisol biospecimens was established. Positive spiritual beliefs was the only R/S variable associated with the peak cortisol awakening response (CAR) ($r_s = .34$; $p = .03$). Poorer physical health was inversely associated with positive spiritual experiences and private religious practices. Poorer mental health was inversely associated with spiritual coping and negative spiritual experiences.

Conclusions—Feasibility, validity, and acceptability of self-collected SDC bio-specimens with an optional mail-back protocol (at moderate temperatures) were demonstrated. Positive spiritual beliefs were associated with neuroendocrine-mediated peak cortisol awakening response activity; however, additional research is recommended.

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Implications for Practice—Objective measures of DSC sampling that include enough collection time points to assess DSC parameters would increase the rigor of future DSC measurement. Breast cancer survivors may benefit from nursing care that includes spiritual assessment and therapeutic conversations that support positive spiritual beliefs. Religiousness, Spirituality, and Salivary Cortisol in Breast Cancer Survivors: A Pilot Study

Breast cancer survivorship is associated with poorer quality of life, impaired physical function, and psychological distress.¹ Chronic stress among breast cancer survivors has been associated with immune dysregulation and risk of breast cancer recurrence.² Moreover, chronic immune dysregulation is influenced by abnormal neuroendocrine activity.³ Following breast cancer treatment, the reported risk of breast cancer recurrence among survivors diagnosed at stages I to III ranges from 8% at 5 years post-treatment and 20% at 10 years post-treatment.⁴

Qualitative reports have suggested that some breast cancer survivors rely on religious practices and spiritual beliefs to cope with stress in survivorship.^{5,6} Based on previous reports linking cancer survivorship with chronic stress, poorer psychosocial outcomes, and risk for chronic neuroimmune dysregulation, a number of studies, including two systematic reviews, have examined neuroendocrine-immune biomarker responses to a variety of psychosocial interventions for cancer populations.^{7,8} Both reviews suggested that comparisons of biomarker outcomes across studies were limited by variations in biomarker measurement and parameter reporting (e.g., cortisol).^{7,8} Additionally, while studies in one review were based on Eastern religious and spiritual traditions, few studies reviewed utilized measures of religiousness and spirituality.⁸ These findings have prompted our interest in exploring relationships between religious and spiritual variables, stress, and health outcomes in breast cancer survivorship; furthermore, these potential relationships are consistent with a psychoneuroimmunological (PNI) framework.

Religiousness, Spirituality, and Health Outcomes

Religious and spiritual variables associated with mental and physical health outcomes⁹ can be generally categorized as: spiritual beliefs (i.e., emotional connectedness to God/higher power), religious practices (i.e., prayer), and congregational support (i.e., social support).¹⁰ These religious and spiritual variables have positive and negative aspects (e.g., positive and negative spiritual experiences) and appear to influence differing aspects of health including self-regulation.^{10,11} However, the literature is generally mixed regarding the nature of relationships between positive and negative religious/spiritual variables and mental and physical health outcomes.

Religion generally refers to denomination affiliation, public religious practice (e.g., attendance), and specific beliefs in religious tenets (e.g., afterlife).¹² Religious practices are culturally-based practices, including prayer and meditation. Religious beliefs are based on behavioral, social, and doctrinal tenets that involve a system of worship.¹³ Religious coping has been associated with better mental health following stressful life events; however, disruption of one's life may cause chronically-ill individuals to question their religious and spiritual beliefs, resulting in negative religious coping.^{14,15} Individuals undergoing medical

rehabilitation have reported poorer perceptions of health associated with negative religious and spiritual experiences (i.e., viewing illness as punishment from God/ higher power).¹⁶

Spirituality refers to an emotional connectedness with the transcendent (i.e., God/ higher power) and may not be associated with formal religion.^{17,18} Positive spiritual experiences and congregational support (i.e., social support) have been associated with better mental and physical health in chronically-ill individuals.¹⁹ Due to overlap in religious and spiritual constructs in the literature, these constructs are commonly denoted as “religious/spiritual.”²⁰

Psychoneuroimmunological (PNI) Model of Health

The PNI model of health seeks to explain relationships between the stress, thoughts and emotions, psychosocial-behavioral components, and neuroendocrine-immune interactions.^{3,21} Psychosocial-behavioral components include: positive and negative aspects of beliefs (e.g., religious and spiritual), personality traits, affect, social support, and coping skills. As the brain perceives stress, personal co-factors (e.g., co-morbidities), “lived experiences,” and psychosocial-behavioral factors influence perceptions of stress and subsequent psychological and physiological responses.^{21,22} Thoughts regarding stress are communicated from the brain to the immune system via neuro-endocrine and hormonal pathways. The adaptive immune system response is a continuum of either wellness or disease vulnerability.²¹

During acute stress, two primary neuroendocrine-immune pathways are activated. The sympathoadrenomedullary axis activates the sympathetic nervous system to release norepinephrine, inducing a “fight or flight” response.²³ A short time after, the hypothalamic-pituitary-adrenocortical axis (HPAA) releases glucocorticoids, primarily cortisol hormone. Cortisol activates macrophages, lymphocytes, and proinflammatory cytokines to address the perceived threat.²⁴ With chronic stress, prolonged HPAA vigilance becomes detrimental, resulting in chronic immune dysregulation and negative health outcomes.^{3,24,25}

Diurnal Salivary Cortisol

Salivary cortisol is commonly used as a proxy measure for HPAA activity in cancer research, as it is favored for reducing the burden of venipunctures.²⁶ Associated with circadian rhythm, the diurnal cortisol rhythm portrays lowest concentrations at night (i.e., the bedtime nadir), slowly rising over the pre-awakening hours, sharply rising to a peak within 30 to 45 minutes after waking (i.e., the cortisol awakening response [CAR]), and declining by 60 minutes after waking for the remaining waking hours of the day (i.e. the cortisol slope).^{27–29}

It is important to note that different parameters provide different information regarding HPAA activity. Due to variability between and among individuals, a single cortisol measurement at one time point has limited interpretive value.³⁰ Similarly, measuring total cortisol production and mean values over 24 hours does not accurately depict HPAA activity, as confounding influences can result in aberrant axis activity.³¹ Two main cortisol

parameters that do consider the diurnal rhythm for clinical outcomes research are the CAR and the cortisol slope.

A certain amount of cortisol is necessary for individuals to manage anticipated daily stress. A sharply rising surge in cortisol associated with the CAR is the expected normal representation of HPA activation upon waking.²⁷ In comparison, cortisol slope is interpreted with respect to patterns of steepness (better health outcomes) versus blunting or flattening (poorer health outcomes). Abnormal CAR and cortisol slope patterns suggest dysregulation of HPA function.²⁸

The CAR is independently regulated compared to other cortisol parameter measures and may be genetically influenced.³² Recent data suggest the CAR is mediated by the suprachiasmatic nucleus via an extra-pituitary pathway to the adrenal system and does not appear to affect cortisol secretion at other time points during the diurnal rhythm.^{29,33} Previously, there was not a consensus on the best way to characterize the CAR parameter; therefore, variances in measuring the CAR exist in the literature. The CAR has been conceptualized as maximum concentration of repeated cortisol measures obtained during the first hour after waking (i.e., the peak CAR).²⁶ The CAR has also been defined as the net change CAR, representing a dynamic change in cortisol concentration from awakening (i.e., “eyes open”) to 30 to 45 minutes after waking.^{27,28} A strong relationship between the peak CAR and the net change CAR has been reported ($r = .68$).³⁴ Alternatively, the CAR has been calculated as the area under the curve with respect to increase (AUCi). This paper focuses on the peak CAR and net change CAR parameters.

Currently, the peak CAR may offer more relevance than the net change CAR for examining relationships between HPA and psychosocial factors.³⁵ The initial sharp rise in the peak CAR has been associated with better psychosocial and physical health.³⁶ Acute stress has been associated with heightened CARs (i.e., CARs higher than cohort average), while chronic stress has been associated with blunted CARs (i.e., CARs lower than cohort average).³⁷ A previous meta-analysis concluded that some psychosocial variables (e.g., job stress and life stress) were associated with peak CARs, while other psychosocial variables (e.g., decreased vitality) were associated with blunted CARs.³⁵ Therefore, both heightened and blunted CARs have been observed with psychosocial stress and poor health outcomes.²⁸

Salivary cortisol sampling protocols have been widely varied in the literature and do not always include timing of sampling with respect to circadian rhythms.³¹ Moreover, published descriptions of salivary cortisol sampling methods are often brief and the feasibility of participant self-collection and storage of salivary cortisol specimens is not clear. Study participation is typically dependent on living in close geographical proximity to the study site, creating a burden of participation for those who wish to participate but live further away. Therefore, the primary aim of this study was to determine the feasibility and acceptability of a salivary cortisol self-collection protocol with a mail-back option for the purpose of examining diurnal cortisol patterns in breast cancer survivors who may be at risk for neuroendocrine dysregulation in extended survivorship. Furthermore, few studies have examined relationships between salivary cortisol and measures of religiousness and spirituality; therefore, a second aim was to examine relationships between and among

religious/ spiritual variables, perceptions of health, and salivary diurnal cortisol parameters, specifically, the peak CAR and the net change CAR. Findings from this study informed the design of a prospective, spiritually-based intervention with diurnal salivary cortisol as an outcome measure.

Method

Design and Setting

This was an observational, cross-sectional pilot study to determine feasibility and acceptability of a salivary bio-specimen self-collection protocol. Feasibility and acceptability were examined by participation and attrition rates, participant feedback, and whether bio-specimens met established criteria for salivary cortisol assay testing. Institutional Review Board approval was obtained from the University of Missouri. Informed written consent was obtained from all participants who were advised participation was strictly voluntary. A mail-back option was offered to participants who would not have been able to participate due to travel burden. All salivary bio-specimens were self-collected by participants in their home settings and were refrigerated until returned to the laboratory within two weeks of collection.

Sample

We invited 72 women who were part of a database of breast cancer survivor participants from our previous studies (N=350+) to participate in this study. Continuous recruitment and enrollment occurred over a one-year period until a final sample of (n=41) was reached. Inclusion criteria included participants who were post-breast cancer treatment for at least three months, diverse breast cancer stages and treatment histories, English-speaking, and able to provide informed consent. In order to control for potential confounders of cortisol, exclusionary criteria included: steroid-based prescription use, nicotine use, dry mouth symptoms, or acute illness on the day of specimen collection.²⁶ Participants were compensated with \$20 gift cards for their time.

Subjective Measures

Demographics—Participants provided population demographics, self-reported breast cancer medical treatment histories, prescription and nonprescription drug regimens, nightly sleep times, and caffeine and alcoholic beverages consumption. Participants completed psychosocial measures (i.e., religiousness/ spirituality and perceptions of health) as relationships between psychosocial variables and a neuroendocrine-mediated biomarker (i.e., cortisol) would further inform PNI variable relationships.

Brief Multidimensional Measure of Religiousness/Spirituality (BMMRS).¹²

The BMMRS is a 38-item, self-report measure of religious and spiritual variables created by expert panel consensus. This study utilized five BMMRS domains: spiritual experiences, spiritual coping, forgiveness, private religious practices, and congregational support. These 5 domains were further divided into seven subscales: positive spiritual experiences (e.g., “I feel God/ higher power’s presence.”), negative spiritual experiences (e.g., “I wonder whether God/higher power has abandoned me.”), forgiveness (e.g., “I know that God/ higher power

forgives me.”), spiritual coping (e.g., “I work together with God/ higher power as partners.”), private religious practices (e.g., prayer practices), positive congregational support (e.g., “If you had a problem or were faced with a difficult situation, how much comfort would the people in your congregation be willing to give you?”), and negative congregational support (e.g., “How often do the people in your congregation make too many demands on you?”). Lower BMMRS subscale scores reflected greater degrees of religious and spiritual experiences. Previous studies have reported acceptable internal consistency for each BMMRS subscale.³⁸

Medical Outcomes Study Short-Form 36 – Version 2 Health Survey (SF-36v2)³⁹

—The SF-36v2 is a 36-item self-administered measure that assesses eight domains of functional physical health and mental health. Electronic scoring of SF-36v2 subscale items was conducted using Quality Metric Health Outcomes Scoring Software (QMHOSS) version 4.5 which calculates and recodes participants’ raw scores on a scale of 0 (poor perceptions of health) to 100 (best perceptions of health).⁴⁰ The QMHOSS then applies T-based scoring to participants’ scores to allow standardization of scores for comparison with 2009 U.S. general population T scores with a reported mean of 50 (10 SD). Therefore, in comparison to the 2009 adult general population, participants’ subscale scores lower than 50 reflected poorer than average perceptions of health; subscale scores higher than 50 reflected better than average perceptions of health.⁴⁰ For all subscales, the SF-36v2 has reported internal consistency ranging from .83 to .95.³⁹

Cortisol—The primary salivary cortisol measure was the peak CAR determined as the maximum cortisol concentration obtained from two salivary cortisol measures: the first measure at time of awakening, and the second measure at the post-awakening peak (i.e., 30 to 45 minutes after waking).^{26,27,30} Post-awakening specimens were obtained starting at 30 minutes after waking; however, it should be noted that the peak CAR may occur between 30 to 45, up to 60 minutes after waking, followed by cortisol decline at approximately 60 minutes after waking.^{26,27} Therefore, specimens obtained from 30 to 60 minutes after waking were useful in determination of the CAR. CAR analyses included the net (or dynamic) change in CAR, defined as salivary cortisol concentration change from awakening to 30 to 45 minutes after waking.^{27,29} Although the CAR parameter was the measure of interest, mean diurnal cortisol concentrations at awakening, post-awakening, and bedtime; and cortisol slope were also examined.

Data Collection and Management

Passive drool specimens were collected at three time points during the waking day (awaken, 30 to 45 minutes after waking, and bedtime) for two consecutive days, which yielded six collection time points.²⁶ Participants were provided detailed written instructions for the data collection procedure including: time/date prompts, food and drink restrictions, avoidance of caffeinated and alcoholic beverages within two hours of specimen collection, and avoidance of brushing teeth within two hours of a specimen collection. Importance of following the collection protocol was emphasized with participants during recruitment. Consistency between self-reported adherence to salivary specimen collection protocols and electronic

monitoring has been reported.⁴¹ Participants completed data collection journals, including dates and times of collection.

Salivary bio-specimen mail-back protocol—Participants refrigerated salivary specimens within two hours of collection and returned specimens to the laboratory within two weeks of collection. Participants were asked to keep specimens cooled during transportation to the lab. Mailed specimens were returned via triple packaging in a bubble-wrap mailer. The average mail time for distance participants was anticipated to range from one to three days given the study site's central state geographical location. Salivary cortisol bio-specimens remain satisfactory for analyses when left at room temperature for up to four weeks.²⁶ Mailed bio-specimens were requested and received during autumn and spring seasons when temperatures were moderate and risk of extreme weather temperatures for the region was low.

Upon laboratory receipt, all salivary bio-specimens were inspected, stored at –20 degrees Celsius, and later transferred to long-term storage at –80 degrees Celsius. Among the 246 salivary bio-specimens received for analysis, 92% (n=225) were acceptable for further analysis after exclusion of salivary cryovials that did not meet assay control parameters, exceeded four standard deviations, or were discolored (n=21).

Data Analysis

Enzyme immunoassay procedures—Saliva cortisol concentrations were measured in each individual saliva sample using a commercially-available salivary cortisol enzyme immunoassay kit (Cat. #1-3002, Salimetrics, Carlsbad, CA). The Salimetrics laboratory protocol was followed. Samples were thawed, vortexed, and centrifuged at 3000 rpm for 15 minutes directly preceding assays. Standards, controls, and samples were pipetted in duplicate on the microtiter plate provided. The diluted enzyme conjugate was added; the plate was mixed for five minutes at 500 rpm, then incubated for 55 minutes at room temperature. The plate was washed four times with 1X wash buffer. The tetramethylbenzidine (TMB) substrate solution was added; the plate was mixed for five minutes at 500 rpm, and placed in the dark at room temperature for 25 minutes. The 3M stop solution was added and the plate was mixed for three minutes at 500 rpm. The plate was read using a plate reader at 450nm; a secondary filter at 490nm was also used. A 4-parameter curve fit was used to calculate the results. Inter-assay variation was calculated from the low and high controls provided by the kit manufacturer. Intra-assay variation was calculated by averaging the coefficient of variation (CVs) of replicate wells from 20 randomly chosen samples. Inter-assay variation for the assays was 5.3% and average intra-assay variation was 4.6%. The assay sensitivity was 0.007 µg/dL.

IBM Statistics SPSS software version 22 (IBM Corp., Armonk, NY) was utilized for statistical analysis. Positive skewness of cortisol values was observed by histogram and by Shapiro-Wilk testing ($S-W = .81$; $p < .001$); therefore, raw cortisol values were logarithmically ($\log_{10}$) transformed for statistical analysis. Descriptive statistics were conducted on all quantitative variables. Tests for normality and goodness of fit of the variables of interest demonstrated skewness and kurtosis; therefore, variable relationships

were examined using Spearman rank correlation coefficients or point bi-serial correlation coefficients, depending upon variable type.

Results

Sample Characteristics

Table 1 provides descriptive statistics of the sample demographics and breast cancer treatment histories. The sample (n= 41) was female, a majority Caucasian (95.1%), and an average age of 66 years. A majority of participants were married (58.5%), reported a Christian (87.8%) faith preference, and resided in a Midwestern urban population (68.3%).

Religiousness/ Spirituality (BMMRS)

A majority of participants reported being moderately religious (63.4%) and moderately spiritual (51.2%) (see Supplement Table 1). Among the sample, 58.4% reported a significant gain in faith, while 22% reported a significant loss in faith. Based on subscale scores, participants reported having above average positive spiritual experiences (14.2 M, 5.5 SD, range 6.0–36.0), fewer negative spiritual experiences (7.9 M, 0.4 SD, range 2.0–8.0), greater forgiveness (4.3 M, 1.3 SD, range 3.0–12.0), and greater spiritual coping (10.9 M, 2.8 SD, range 4.0–20.0). The majority of participants reported private religious practices at least once per week (19.6 M, 8.0 SD, range 5.0–37.0). Participants reported higher than average positive congregational support (2.9 M, 1.6 SD, range 2.0–8.0); and few experiences of negative congregational support (7.3 M, 1.0 SD, range 2.0–8.0). Congregational support data were missing from 5 participants who did not answer these items.

Health Outcomes (SF-36 version 2)

Scores range from 0 to 100, with higher scores indicative of better perceptions of health (see Supplement Table). Based on subscale rankings, participants reported average overall physical health (50.4 M; 10.0 SD) and slightly higher than average overall mental health (55.0 M; 10.0 SD) compared to 2009 U.S. general population normative data.⁴⁰ Highest subscale scores among the sample were observed for general (physical) health (54.8 M; 10.0 SD) and vitality (energy) (54.8 M; 10.0 SD); and these scores were slightly above 2009 general population normative data.

Salivary Cortisol

Descriptive statistics of raw salivary cortisol measures are presented in Table 2. The sample range for peak CAR was .05 µg/dL to 1.62 µg/dL; the mean peak CAR was .41 µg/dL. Examples of selected participants' diurnal cortisol patterns are presented in the Figure. Definitive conclusions could not be drawn from individuals' diurnal cortisol patterns due to lack of clinical context and small sample size.

Feasibility, Validity, and Acceptability

This study had 100% participation from enrolled participants with zero attrition. Completed journal data demonstrated participants' understanding of following the data collection protocol. Anecdotal feedback suggested collection of passive drool was burdensome for a

few participants with respect to dry mouth and time. Journal data revealed that participants' required an average 10 minutes at the awakening time point to collect an adequate volume of passive drool for one cryovial.

The majority of participants ($n=29$) (70.7%) transported salivary bio-specimens to the lab. Among personally-delivered bio-specimens, two (6.9%) participants' data were excluded due to missing time point data. Among bio-specimens mailed back ($n=12$) (29.3%), one (8.3%) participant's data were excluded due to insufficient specimen volume necessary for analysis. Validity of mailed cortisol bio-specimens was assessed by comparing mean salivary cortisol concentrations between self-transported specimens (.28 M; .03 SD $\mu\text{g/dL}$) and mailed specimens (.28 M; .04 SD $\mu\text{g/dL}$).

Average duration of sleep reported during participation days was 8 hours and 59 minutes. Per participant journals, average awakening bio-specimens were collected at 6:45 a.m. (1:33 SD) (hours: minutes); post-waking bio-specimens were collected at 7:24 a.m. (1:33 SD); and bedtime bio-specimens were collected at 9:46 p.m. (2:35 SD).

Spearman's rho Correlations between Subjective Measures and Salivary Cortisol

Spearman's rho correlations between the subjective measures and salivary cortisol are presented in Table 3. There were no statistically significant relationships between demographic variables and salivary cortisol measures. Positive spiritual experiences was the only spiritual variable to demonstrate a statistically significant relationship with the peak CAR ($r_s = .34$; $p = .03$); however, the relationship was small. Small relationships were also observed between the peak CAR and private religious practices, as well as negative congregational support; however, these results were not statistically significant. Similarly, small relationships were observed between the net change CAR and forgiveness, private religious practices, and spiritual coping (Table 3). There were no statistically significant relationships between SF36v2 (perceived) health outcomes and salivary cortisol measures.

Spearman's rho Correlations between BMMRS and SF36v2 Variables

Spearman's rho correlations between religious/spiritual variables and health outcomes variables are offered in Supplement Table 2. Positive spiritual experiences demonstrated low inverse associations to all physical health subscales. However, only relationships between positive spiritual experiences and physical function ($r_s = -.39$; $p = .01$) and bodily pain ($r_s = -.37$; $p = .02$) were statistically significant. Associations between positive spiritual experiences and mental health variables were not statistically significant.

Forgiveness did not demonstrate a statistically significant relationship with any physical or mental health subscales. Negative spiritual experiences were inversely associated with all physical health and mental health subscales. A moderate association was observed between negative spiritual experiences and emotional role-function ($r_s = -.48$; $p = .00$) and a low association was observed with vitality (i.e., energy) ($r_s = -.32$; $p = .04$). Remaining associations between negative spiritual experiences and mental and physical health subscales were not statistically significant.

Spiritual coping was inversely associated with all physical health and mental health subscales. Low to near-moderate associations were observed between spiritual coping and vitality ($r_s = -.42$; $p = .01$); social function ($r_s = -.37$; $p = .02$); and emotional role-function ($r_s = -.31$; $p = .05$). Low to near-moderate relationships were demonstrated between spiritual coping and physical function ($r_s = -.30$; $p = .05$) and bodily pain ($r_s = -.40$; $p = .01$).

Private religious practices (i.e., prayer) demonstrated an inverse association with all physical health and mental health variables with the exception of the mental health subscale (i.e., emotional well-being). Moderate associations were observed between private religious practices and social function ($r_s = -.44$; $p = .00$); physical function ($r_s = -.53$; $p = .00$); physical role function ($r_s = -.45$; $p = .00$); and bodily pain ($r_s = -.48$; $p = .00$).

Positive congregational support demonstrated positive associations with all physical health and mental health subscales. Statistically significant relationships were observed between positive congregational support and mental health ($r_s = .40$; $p = .02$) and between positive congregational support and vitality ($r_s = .34$; $p = .04$).

Negative congregational support demonstrated low inverse relationships with both mental health and physical health subscales. However, only the associations between negative congregational support and emotional role-function ($r_s = -.35$; $p = .04$); and physical function ($r_s = -.34$; $p = .04$) were statistically significant.

Discussion

This pilot study demonstrated feasibility of a salivary cortisol collection protocol in the home setting which allowed biospecimen collections to be synchronized with diurnal rhythms. Although passive drool and oral synthetic swab collections are both acceptable for salivary cortisol collection, data suggested passive drool collection could take longer than anticipated, potentially risking a discrepancy between the intended collection time and the next time point along the diurnal rhythm. Assessment of the CAR is time-sensitive, as salivary cortisol concentrations change significantly in increments from waking through the first 30 to 45 minutes after waking, declining by 60 minutes after waking. Differences observed in participants' line graph data noted in the Figure may be possibly attributed to variability in passive drool collection times, as well as variances between and within participants.

Self-collection of biospecimens is ideal for diurnal cortisol measurement, as it allows collection in the natural home setting, which is more likely to capture participants' true diurnal patterns compared to the laboratory setting. Anecdotal findings suggested greater participant burden occurs with passive drool collection. Therefore, use of an oral, polymer-based swab, which requires two to five minutes for adequate saturation and holds 200 to 1000 μL is suggested for future cortisol studies when the diurnal measure of interest is time-point sensitive.⁴²

The pilot study successfully demonstrated validity of salivary cortisol biospecimens obtained through a mail-back protocol. These findings inform future research design in which recruitment and participation would be otherwise limited due to participant burden with

respect to travel time and scheduling. Acceptability was confirmed with 100% participation of individuals who were consented. This suggests that breast cancer survivors would be interested and amenable to participation in studies using a self-collection protocol.

An underlying assumption of the study design was that individuals previously treated for breast cancer may have neuroendocrine dysregulation (e.g., blunted CAR pattern) in survivorship. Although peak CAR and net CAR reference ranges for individuals post-treatment for breast cancer have not been established, mean morning and evening cortisol concentrations were observed within the expected ranges reported for healthy adults.⁴³ Examination of line graph data suggested that CAR patterns were blunted; however, without clinical context, the etiology of these patterns remain unclear. Comparison of CAR data with measures of participants' anticipatory daily stress would add further information in future study.

With respect to the CAR, data suggested the peak CAR may have more utility than the net change CAR for investigating relationships between HPA activity and religious/spiritual variables. However, given participants' perceptions of being in generally good health, HPA activity may have been normal which may have limited the detection of abnormalities in the peak CAR and the net change CAR. While this study did not demonstrate an association between the net change CAR and religious/spiritual variables or health outcomes variables, a larger sample size may offer further insight into these potential relationships. Furthermore, unreported deviations from sampling protocol instructions may have negatively influenced the detection of the net change CAR. Alternatively, the net change CAR may not correspond to subjective measures. A recommendation for future salivary cortisol study design would be to include electronic monitoring of self-collection sampling time points as well as the number of minutes required to complete each self-collection.

Relationships between Subjective Measures and the CAR

This was a (self-reported) moderately religious and moderately spiritual sample. One of the aims of the study was to examine whether religious/spiritual beliefs demonstrated an association with cortisol production in breast cancer survivors potentially at risk for chronic stress and neuroendocrine-immune dysregulation. Our data suggest positive spiritual beliefs may influence the peak CAR, although further study is needed to understand this relationship. With the exception of positive spiritual beliefs, BMMRS findings were similar to those reported by Ward⁴⁴ who found no significant association between BMMRS religious/spiritual variables and the CAR. Further investigation of these findings may provide clarification of these relationships.

Population demographic variables were not associated with any salivary cortisol parameters. It was anticipated that older age could be associated with less healthy cortisol patterns, although high intra-individual variances in diurnal cortisol have been reported.⁴⁵ Participants in this study were an average nine years post-treatment for breast cancer and in good health; therefore, their HPA activation may have been normal. Longitudinal measures are recommended to further examine diurnal cortisol variability between and among participants.

Pilot data did not support a relationship between perceptions of health and CAR measures. Participants reported perceptions of good health; therefore, cortisol findings may be attributed to other confounders (e.g., specimen collection timing), rather than indicative of negative health outcomes. Electronic time/stamp measures may help clarify the nature of participants' adherence to the study protocol. Finally, participants may have perceived their own health as satisfactory compared to peers in ill health. Importantly, these perceptions may not correspond to clinical measures.

Relationships between Religious/ Spiritual Variables and Perceptions of Health

The most interesting associations between religious/spiritual variables were physical health with positive spiritual experiences and private religious practices, in which small and moderate-sized relationships were observed, respectively. It was not surprising that poorer physical health was associated with greater degrees of positive spiritual experiences (i.e., loving God/ higher power) and more frequent private religious practices (i.e., prayer). Prayer and meditation may be ways to emotionally connect with God/ higher power. In the Judeo-Christian faith traditions, a close relationship with God is viewed as essential for recovery and curing of disease.⁴⁶ While previous reports have been mixed, our findings are consistent with previous findings in which better physical health outcomes were associated with positive spiritual experiences and prayer.^{17,47}

Poorer mental health was associated with greater degrees of spiritual coping (i.e., relying on God/ higher power) and greater frequencies of negative spiritual experiences (i.e., punitive God/ higher power). These findings support previous reports in which negative views of God were associated with disease progression.⁴⁸ Similar to previous literature in which positive social support was associated with better mental health in ovarian cancer survivors⁴⁹, our data demonstrated positive congregational support was associated with better mental health.

Implications for Nursing

Self-collection of salivary cortisol in the home (i.e., natural) setting is a viable and feasible research protocol. Importantly, this protocol allows synchronization of salivary cortisol awakening measures with circadian rhythm which increases accuracy of conclusions regarding findings. However, the degree of adherence to the protocol is dependent on self-report; therefore, objective measures (e.g., electronic bottle caps) are suggested for future research.

Our data further inform diurnal salivary cortisol parameter ranges for breast cancer survivors in extended survivorship. However, future research should include at least four or five collection time points per day (i.e., awaken, 30 minutes postwaking, 45 minutes postwaking, 60 minutes postwaking [optional], and bedtime) and across at least two or more days in order to further establish diurnal salivary cortisol parameters, including the CAR. Oral synthetic swabs to minimize the burden of time required for specimen collection at each sampling time point and objective measures of salivary collection times (i.e., electronic bottle caps) would enhance the measurement rigor of the diurnal salivary cortisol rhythm.

Nurses should be aware that although religiousness and spirituality are two separate constructs, breast cancer survivors may use these terms interchangeably as definitions

commonly overlap. Because religiousness and spirituality are associated with perceptions of mental and physical health for some individuals, breast cancer patients may benefit from nursing support of positive spiritual beliefs and private religious practices. Specifically, nurses should be prepared to assess patients' religious and spiritual needs and anticipate ways to engage in therapeutic conversations that support patients' religious and spiritual beliefs.

Limitations

This pilot study was underpowered, cross-sectional in design, and had a small sample. The sample was comprised of female, predominantly-white, and older breast cancer survivors which limits generalization of findings. Moreover, given the exploratory aim of the pilot study with a focus on feasibility and acceptability, subjective measures of stress were not included with the assessment of salivary cortisol. A larger sample with more recently treated breast cancer survivors using measures of anticipatory daily stress may allow detection of distinct relationships among stress and salivary cortisol production.

Participants reported average general health and were an average of 9 years post-treatment for breast cancer. Therefore, HPA activity may have been preserved, limiting the ability to detect abnormalities diurnal salivary cortisol parameters. This sample utilized three collection time points per day, which potentially limited detection of both CAR parameters. Furthermore, the average awakening bio-specimen required 10 minutes to collect, which may have limited characterization of the CAR. Objective measures of data collection times were not used and unreported delays in specimen collections may not allow true depiction of the diurnal cortisol rhythm. Similarly, while attempts were made to control for potential known confounders of cortisol concentration (e.g., caffeine consumption prior to specimen self-collection), unreported deviations from the protocol could have potentially influenced salivary cortisol production.

Conclusion

Feasibility, validity, and acceptability of self-collected salivary cortisol bio-specimens with an optional mail-back protocol (at moderate temperatures) were demonstrated in this sample of breast cancer survivors. Perceptions of mental and physical health were not associated with either CAR parameter. Positive spiritual beliefs were associated with the peak CAR, but not with the net change (dynamic) CAR. Further evidence is recommended to clarify these relationships.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviation: CAR, Cortisol Awakening Response

Figure. Examples of Selected Participants' Diurnal Salivary Cortisol Patterns

Line graphs of five participants represent common patterns of variability in salivary cortisol diurnal rhythms observed in the data. Raw cortisol values are represented on the y-axis in $\mu\text{g/dL}$ (micrograms/deciliter) for ease of comparison to current literature. The x-axis represents a 24 hour period, starting at 0:00 (12:00 am) through 24:00 (midnight). Line graphs represent the mean cortisol concentrations collected at awaken, 30 minutes after waking (peak CAR), and bedtime for two consecutive days for each of the five participants. The entire sample peak CAR mean was .41 $\mu\text{g/dL}$ and is marked with a horizontal line. A participants' line graph with a peak CAR pattern similar to the sample peak CAR mean is denoted as well. Blunted peak CARs, heightened peak CARs (i.e., exaggerated peaks), and flattened slopes may represent abnormal diurnal cortisol production, although further study is necessary to clarify the relationship between pattern variability and health outcomes in breast cancer survivors.

Table 1

Descriptive Statistics of Sample Demographics

Variable	Frequency (n=41)	Percent (%)	Mean	SD
Age Range: 51–88 years			66.27	8.94
Post-Treatment for Breast Cancer Range: 1–21 Years			9.23	4.04
Race				
African American	2	4.90		
Caucasian	39	95.10		
Marital Status				
Single	3	7.30		
Married	24	58.50		
Widowed	5	12.20		
Divorced	1	2.40		
Missing Data	8	19.50		
Religious Preference				
Protestant	24	58.50		
Catholic	9	22.00		
Jewish	1	2.40		
Non-denominational	3	7.30		
None	2	4.90		
No answer	2	4.00		
Breast Cancer Stage				
0	4	9.80		
I	7	17.10		
II	14	34.10		
III	3	7.30		
IV	3	7.30		
Unknown	10	24.40		
Recurrence of Breast Cancer				
Yes	4	9.80		
Secondary Cancer				
Yes	11	26.80		

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Variable	Frequency (n=41)	Percent (%)	Mean	SD
Lymphedema				
Yes	11	26.80		
History of Smoking				
Yes	7	17.10		
History of Radiation				
Yes	30	73.20		
History of Chemotherapy				
Yes	29	70.70		
SD = Standard Deviation				

Table 2

Descriptive Statistics of Salivary Cortisol Measures^a

Variable	(n)	Minimum (µg/dL)	Maximum (µg/dL)	Mean (µg/dL)	SD (µg/dL)
Cortisol Awaken	40	.07	.89	.31	.18
(Awaken Reference Range for Healthy Adult) ^b		<i>b</i> .09	<i>b</i> 1.55		
Cortisol Post-Awaken	40	.05	1.62	.41	.28
Cortisol Bedtime	39	.02	.61	.09	.11
(Bedtime Reference Range for Healthy Adult) ^b		ND	<i>b</i> .36		
Peak CAR ^c	40	.05	1.62	.41	.28
Net Change CAR ^d	40	-.22	1.20	.10	.25
Diurnal Cortisol Mean	41	.03	.98	.27	.16
Cortisol Slope (µg/dL/h) ^e	36	-.07	.00	-.02	.02

Abbreviations: SD = standard deviation; µg/dL = microgram per deciliter; ND = not determined; CAR = cortisol awakening response; µg/dL/h = microgram per deciliter per hour.

^aRaw salivary cortisol measures.

^bAardal & Holm⁴³

^cMaximum (i.e., peak) cortisol measure obtained 30 to 60 minutes after waking.

^dMean cortisol concentration net change from awakening to 30 to 60 minutes after waking.

^eDecline in cortisol concentration from peak CAR to bedtime across waking hours.

Table 3
Spearman's rho Correlations between BMMRS and Salivary Cortisol (log) Measures

Variables	(n)	Peak ^a CAR	Net Change ^b CAR	Mean ^c Diurnal Cortisol
BMMRS Religiousness/Spirituality				
Positive Spiritual Beliefs	40	.34	.06	.24
Negative Spiritual Beliefs	40	.00	.11	.15
Forgiveness	40	.10	.25	-.06
Spiritual Coping	40	-.15	-.14	-.10
Private Religious Practices	40	.25	-.18	.21
Positive Congregational Support	35	-.05	.06	-.01
Negative Congregational Support	35	-.27	.00	-.29

Abbreviations: BMMRS = Brief Multi-dimensional Measures of Religiousness/ Spirituality; CAR = cortisol awakening response.

^aPeak cortisol measure obtained 30 to 60 minutes after waking.

^bMean cortisol concentration net change from waking to 30 to 60 minutes after waking.

^cCalculated as the two day mean cortisol of all 6 time points.

^dp < .05