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GME Study

Gene Expression, Meditative Movement, and Emotional Distress (GME)

NCT04213872

Study Protocol and Statistical Analysis Plan

2. Method

This was a single-group pilot study testing the effects of 8 weeks of MM practice on changes in cognitive function, anxiety, sleep quality, depression, and selected gene expression factors. The enrollment goal was to consent and assign forty BCSs to an eight-week MM program. Measures on cognitive functioning, associated symptoms/conditions, and gene expression data were collected before and after the 8-week MM program. This study utilized the Paired Sample t-test of SPSS [22] to report p-values from the analysis of pre- and post-MM intervention changes in the cognitive functioning, associated symptoms/conditions, and gene expression data. This study was approved by both the IRB committees at Pomona Valley Hospital Medical Center (PVHMC as the primary IRB) and at Arizona State University (ASU as the secondary IRB).

2.1 Study Population

2.1.1 Eligibility Criteria

Inclusion criteria: minimum 45 years of age; female patients diagnosed with breast cancer, stages 0–III; between six months and five years past primary treatment; ability to speak or understand English; and post-menopausal for at least one year. Exclusion criteria: Women who were unable to stand (e.g., wheelchair or walker bound); patients who were too weak or ill; patients on antibiotics; women working on night shift; and patients with anemia or uncontrolled diabetes. Pregnant women, mentally disabled persons, and prisoners were excluded.

2.1.2 Participant Recruitment

On a daily basis, the clinical trials research coordinator (CRC) at the Pomona Valley Hospital Medical Center (PVHMC) Cancer Care Center (CCC) in Pomona, CA, identified potential participants with the support of site oncologists, site oncology nurses, and the site cancer registry. Flyers were displayed at the Breast Cancer Care Center at the CCC and other affiliated breast cancer care centers in the region. The site cancer registry prepared a mailing list based on the eligibility criteria and invitations were mailed. The CRC generated a list of potential participants based on referrals from the CCC staff and from phone calls from potential participants responding to flyers and mailings.

2.1.3 Screening and Consenting

There were two options for screening and consenting potential participants. In the first option, the site PI or the CRC contacted potential participants referred by the oncology team to screen for study interest and eligibility. In the second option, potential participants responded to the mailed invitation sent by the CRC. Study eligibility and enrollment for participants recruited and screened through both options were confirmed upon review of their medical records after obtaining written consent from potential participants in accordance with Good Clinical Practice (GCP) and Health Insurance Portability and Accountability Act of 1996 (HIPAA). As CCC staff members, the CRC and site PI had access to medical records. After reception of signed Informed Consent form and medical chart review, study participants were scheduled to attend the MM classes.

2.2 Study Intervention

2.2.1 The Intervention

The MM (Qigong/Tai Chi Easy) intervention is a standardized, protocol [23] with a formal training program for practice leaders from the Institute of Integral Qigong and Tai Chi (IIQTC) and has been used in previous research with various populations [8, 24, 25]. This practice is similar to the short, simplified forms used in the majority of Tai Chi research protocols showing health benefits [26]. The protocol is taught as a series of repeated and simple-to-learn movements rather than long chains of choreographed moves that are more difficult to learn (typical of how traditional long-form Tai Chi is taught).

In this study, the MM intervention was implemented over 8 weeks with class sessions once a week at the Pomona Cancer Care Center. Each class session was approximately one hour. The participants learned gentle

movements, ranging from mild to moderate levels of exertion. The participants were asked to practice the MM exercises at home, at their own pace, most days of the week, totaling at a minimum, 2 ½ to 3 hours per week and to log their MM practices in a logbook provided to them. A professionally produced DVD and manual demonstrating a core set of 10, and additional exercises for variety were given to participants to help guide their practice at home. The lead investigator, a certified Mind-Body Medicine Practitioner, and certified QG/TCE practice leader assisted the research participants with the MM sessions. The PI and CRC provided support and guidance to the participants during the MM program.

2.3 Measures

Basic demographic data (gender, ethnicity/race, and age) was collected at baseline. Self-report data on cognitive abilities (perceived and objective assessments), anxiety, depression, and sleep quality, and blood draws to examine gene expression, were collected pre- and post- 8-week MM program (within two weeks prior to and subsequent to the intervention).

2.3.1 Cognitive Function (CF) and Cognitive performance (CP) Tests

Cognitive function was assessed using both a self-report and an objective performance test.

Self-reported CF was assessed using the Functional Assessment of Cancer Therapy-Cognitive Function (FACT-COG), 37 items, validated, including 4 subscales including perceived cognitive impairment (PCI), perceptions of effects of cognitive function on quality of life (QOL), and perceived cognitive abilities (PCA) and comments from other (OTH [27-29]

CP Tests: Two brief measures of attention/working memory from the Wechsler Adult Intelligence Scale (WAIS-III) Third Edition [30, 31] were used to assess CP: Digit Span and Letter-Number Sequencing, with reliability ratings of .90 and .82 respectively.

2.3.2 Profile of Mood States Short Form (POMS-SF)

The POMs-SF consists of 37 items, adjectives scored on a 5-point Likert scale [32]. The POMS is one of the most frequently used and validated scales in studies of psychosocial interventions with BCSs, and has been validated with Hispanics [33] and multicultural populations [34]. POMS consists of the Total Mood Disturbance (TMD) dimensions (tension-anxiety; depression-dejection; anger-hostility; confusion-bewilderment; Cronbach's alpha = .93). This pilot study reported results for the tension-anxiety and depression-dejection TMD dimensions (12 items).

2.3.3 Sleep Quality

The Pittsburgh Sleep Quality Index (PSQI): 19 items assess sleep, including subscales for subjective sleep quality, sleep latency, sleep duration, sleep disturbance, habitual sleep efficiency, daytime dysfunction and use of sleep medications [35]. A global PSQI score >5 distinguishes good from poor sleepers with 89.6 % sensitivity and 89.5% specificity and demonstrates Cronbach's $\alpha = 0.83$ [36].

2.3.4 Process Control, Manipulations and Fidelity

Each participant received a phone call or email midweek between class attendance (whether they attended or not) to remind them to document practice time (session/minutes) and weekly record level of exertion. MM Practice Logs were provided for participants to document home practice. The midweek contact served both as process control evaluation and as a method for encouraging adherence. Whenever a participant missed a scheduled class, she received an additional call to encourage adherence.

2.3.5 Gene Expression Data

The PAXgene Blood RNA Tube (Catalog No. 762165) was at room temperature (18-25C) and properly labeled for patient identification. The CRC collected one 2.5 ml of blood into one tube using the standard technique for BD Vacutainer® Evacuated Blood Collection Tubes.

2.3.6 After Blood Collection

The CRC inverted the PAXgene Blood RNA Tube 8 to 10 times and stored the PAXgene Blood RNA Tube upright at room temperature (18°C–25ºC) for a minimum of 2 hours and a maximum of 72 hours before processing or transferring to refrigerator (28°C) or freezer (−20°C). After 48 hours in freezer (−20°C) the blood samples were transferred and stored in a freezer (-80°C) until they were prepared for RNA sequencing. Transport Collected Blood Samples: Blood samples were transported to SC2 Core lab for processing and library preparation for RNA sequencing.

2.4 Retention Strategies

The PI and CRC maintained contact with the study participants by telephone, in person, or email to encourage retention in the study. In addition, to providing log-book instructions and meeting time reminders during this contact, the CRC or PI encouraged questions and sharing on how they are experiencing the research study with goal of establishing a therapeutic alliance between the research staff and the study participants. The study participants were also encouraged to initiate contact with the PI or CRC with any questions or concerns by phone, email, or in person.

2.4.1 Sample Size Justification

The enrollment goal of 40 participants followed the guidelines of Browne [37] to formulate samples sizes for pilot studies. This was the target for recruitment with an expected attrition rate of 20%. Although 27 participants were consented, only 14 completed the study. The 14 who completed the study met the standards established by Julious [38] for pilot study sample sizes. Browne and Julious [37, 38] provided sample size calculation guidelines for pilot studies designed to collect preliminary data for a clinical trial.

2.4.2 Hypothesis Testing Data Analysis Plan

This study utilized the Paired Sample t-test of the SPSS statistical program to analyze the primary outcome changes in cognitive function anxiety, depression, and sleep quality over an 8-week period. Changes in BDNF, NF-kB1, and TP53 gene expression results were analyzed using the paired sample t-test in SPSS to determine if the means of the pre- and post-MM gene expression data were significantly different.

2.4.3 Missing Data

Participant responses were reviewed for missing data and participants were given an opportunity to complete overlooked questions or indicate a preference not to answer during the data collection session. All analyses were conducted on participants who completed the intervention. If post-intervention data was unavailable, missing data were not imputed. As a small pilot study to detect trends in change from pre- to post-intervention, we do not expect there to be a systematic bias introduced by the data loss of a small number of participants. One study participant did not complete her post-MM program WAIS-III measure and did not submit her MM logbook due to schedule conflicts.

2.4.4 Secondary Outcomes

Peripheral blood samples were collected before and after the 8-week MM program. The gene expression data was processed and analyzed by the bioinformatics team at the Single-Cell, Sequencing, and CyTOF Core (SC2), Children's Hospital Los Angeles (CHLA), Los Angeles. These data were analyzed using the paired sample t-test in the IBM SPSS Statistics program.

RNA sequencing: Sequencing libraries were prepared from previously purified RNA using the Illumina TruSeq Stranded mRNA Library Prep kit following the manufacturer’s instructions. Sequencing was performed on a NextSeq 500 platform using 2×75bp chemistry [39].

Gene Expression data analysis: Quality control and adapter trimming was performed using trim galore (v0.4.2) with default parameters [40]. Reads were aligned to the GRCh38 reference genome and transcriptome using HISAT2, v2.1.0 [41], and transcript quantification was performed using featureCounts, v1.5.1 [42]. Differential expression analysis was performed using the ‘DESeq2’ R package, v1.16.1, [43] and a rank score calculated

as -log10(q-val)*sign (log2 FoldChange) was used as input to the GSEA Preranked tool for pathway analysis [44].

The DESeq2 data were used to identify the gene expression changes over the 8-week MM program. DESeq2 (differential expression sequence) files are TMM (weighted trimmed mean of the log expression ratios - trimmed mean of M values) normalized count per million reads.