Study Protocol and Statistical Analysis Plan

Title of Study: Effects of Transcutaneous Vagus Nerve Stimulation (taVNS) in Individuals with Primary Dysmenorrhea: A Randomized Controlled Study
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Study Protocol

Objectives:

This study aims to investigate the effects of transcutaneous auricular vagus nerve stimulation (taVNS) on pain, functional and emotional symptoms, and physical performance in individuals with primary dysmenorrhea (PD).

Design:

A small randomized controlled trial design was conducted online at two time points: 1) baseline/pre-test and 2) post-test (one week after pre-test). Data were collected by face-to-face interview method.

Participants will be randomly assigned to one of two conditions: 1) intervention (women received taVNS treatment) or 2) sham (Sham applications were applied using a current-free headset specifically designed for this purpose. Participants observed the device's functionality but supplied no current).

The study was approved by the Artvin Çoruh University Rectorate Scientific Publications Ethics Committee (date: 18/09/2023, procedure number: E-105136) and was by the ethical standards of the World Medical Association (Declaration of Helsinki).

Methods:

Sample and Recruitment. Women who had never given birth before participated in the study. To participate in the research, the following criteria must be met: 1) being diagnosed with PD according to the No. 345 Primary Dysmenorrhea Consensus Guidelines; 2) an NRS score of at least three during menstruation before treatment; 3) being a woman between the ages of 18-40; 4) a regular menstrual cycle of 28±7 days; 5) being nulliparous; 6) no history of brain surgery, tumor, or intracranial metal implantation; 7) No chronic genitourinary infection or alcohol or drug use. Potential participants were invited to study at Artvin Çoruh University through voice announcements and brochures. Following diagnosis with primary dysmenorrhea at Artvin State Hospital, those who met the study's inclusion criteria received treatment at Artvin Çoruh University Physiotherapy Laboratory between November 2023 and January 2024.

Procedure. The current of taVNS was applied bilaterally from the tragus and turbinate parts of the ear with the VagustimTM device. The current frequency used in modulation mode is 10 Hz, and the pulse width was 300 μs (In modulation mode, the pulse rate and width are automatically changed in a loop pattern. The pulse width was reduced by 50% from its original setting in 0.5 seconds; then, the pulse rate was changed in 0.5 seconds, reduced by 50% from the original setting. Total cycle time is 1 second.). The current intensity was applied for 5 minutes, keeping the current constant where the participant felt comfortable. Sham applications were applied using a current-free headset specifically designed for this purpose. Participants observed the device's functionality but supplied no current.

Measures.

Numerical Rating Scale (NRS). Data was queried separately for lower abdominal, waist, and bilateral thigh pain. Participants were required to complete an NRS questionnaire through the Google Forms web survey platform (Google LLC, Mountain View, CA, USA) before bed at

night on days 1, 2, and 3 of two consecutive menstrual cycles. The questionnaire was based on the most severe pain they experienced during the day. The minimum score of the NRS is 0, and the maximum score is 10. Higher scores are associated with more severe pain intensity.

Pressure Pain Threshold (PPT). Fabrication Enterprises, Inc., used a Push-Pull Force Gauge® (1200-304) to measure the pressure pain threshold. This handheld algometer allows linear force application ranging between 0 and 10 kg (22 lb × ¼ lb and 10 kg × 100 g). Initially, two exercises were performed on the dominant forearm's extensor muscles to familiarize patients with the equipment. The evaluator positioned the algometer perpendicular to each point and applied gradual pressure at a constant rate of approximately 0.5 kg/cm2/s. The pressure was increased until the participant reported feeling pain. Each point was evaluated three times, and the average value was recorded. The measurement was performed on five reference points in the abdomen and one reference point in the lumbar spine, as in a previous study where pain was evaluated in PD.

Menstrual Symptom Questionnaire (MSQ). The scale consists of 22 items and uses a 5-point Likert-type format. Participants were asked to rate the severity of their menstrual symptoms on a scale of 1 (never) to 5 (always). The scale contains three subscales: 'Negative Effects/Somatic Complaints' (items 1-13), 'Menstrual Pain Symptoms' (items 14-19), and 'Coping Methods' (items 20-22). The maximum score on the scale is 110, and the minimum score is 22. MSQ score is calculated by taking the average score of all items in the scale. High scores are associated with increased menstrual symptoms.

Functional and Emotional Measure of Dysmenorrhea (FEMD). Scale consisting of 14 items, evaluates dysmenorrhea functionally and emotionally with a 5-point Likert-type item. The reliability and validity study of the scale developed in 2012. As the scores obtained from the scale with a minimum score of 14 and a maximum score of 70 increase, the functional and emotional impact of dysmenorrhea also increases.

Hamilton Anxiety Scale (HAM-A). Patients with chronic pain often experience mood disorders, such as depression and anxiety. The Hamilton Anxiety Scale (HAM-A) was used to assess the intensity of anxiety symptoms. The scale measures both mental and physical anxiety with 14 symptom-defined items. Each item is scored from 0 (not present) to 4 (severe). The total score range is 0-56. On the anxiety scale, scores of 17 or below indicate mild anxiety, while scores of 18-24 indicate moderate anxiety, and scores of 25-30 indicate severe anxiety.

6-Minute Walk Test (6MWT). The 6-Minute Walk Test (6MWT) was evaluated submaximal functional capacity. 6MWT measures the maximum distance participants can walk as quickly as possible for six minutes.

Positive and Negative Affect Schedule (PANAS). For taVNS administration, the positive and negative affect was assessed before and after treatment using the Positive and Negative Affect Schedule (PANAS). This 5-point Likert-type scale was developed in 1988 and includes 20 items: 10 positive and ten negative. A score range of 10-50 assesses both positive and negative effects.

Statistical Analysis Plan

IBM SPSS version 28.0 software (IBM Corp., Armonk, NY, USA) was utilised for statistical analysis. Before the statistical analysis, the Shapiro-Wilk normality test was used to assess data distribution because of $n<50$. Demographic data and clinical baseline variables were compared among the 2 groups by independent samples t-test for continuous variables and a $\chi 2$ test for categorical variables. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were reported as number and frequency. The independent and paired samples t-test was compared to normally distributed variables between two groups. Two-way analysis of variance (ANOVA) was used for multiple comparisons of normally distributed

variables. The effects of stimulation on pain, pressure pain threshold, menstrual symptoms, and physical performance were calculated using a mixed ANOVA, where the dependent variables were pain, pressure pain threshold, menstrual symptom, and physical performance score, and the independent fixed variables were time, stimulation group (taVNS and sham), and interaction term was group vs time. Post hoc comparisons were carried out when appropriate using Bonferroni correction for multiple comparisons. Greenhouse–Geisser correction was applied when the assumption of sphericity was violated. In the F tests, partial eta squared ($\eta 2$) was used as an effect size indicator, elucidated as small, 0.01; medium, 0.06; and large, 0.14. In the t-tests, Cohen's d (d) was used as an effect size indicator, elucidated as small, 0.2; medium, 0.5; large, 0.8. A p value of <0.05 was considered statistically significant.