ClinicalTrials.gov Submission Cover Page

Institution / Sponsor Name: _____Azusa Pacific University_____

Official Study Title:

The effect of vaginal somatovisceral pain on hip extension mobility and strength in women at mid-cycle with dysmenorrhea vs. controls: A case-control study

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Sponsor / Collaborators:

NA

Methods

Participants

Sixty healthy, non-pregnant female participants aged 18–36 will be recruited from Azusa Pacific University and Mount Saint Mary's University, and via a social media account not associated with any research team member. All participants will complete a phone screen with the primary investigator before the initial visit. The experimental group will consist of at least 30 subjects with a pain rating during menstruation greater than or equal to 4 on a visual analog scale (VAS). The control group will consist of at least 30 age-matched participants with a pain rating less than or equal to 3 during menstruation. Inclusion criteria include regular menstrual cycles (between 27 and 34 days) and no known medical conditions. All participants will be provided written informed consent, and the Azusa Pacific internal review board will approve the study. Participants will be provided informed consent prior to participation.

Exclusion criteria included a history of chronic pain, currently pregnant, having given birth within the previous 6 months, currently breastfeeding, or undergoing in-vitro fertilization treatments. Those with a history of abdominal or pelvic surgery in the last 3 months will be excluded, as well as those with a history of pelvic pathology, including interstitial cystitis, endometriosis, pelvic inflammatory disease, and irritable bowel syndrome. Women with a history of sexual assault will be excluded, as well as those who are anovulatory without hormonal BC or amenorrheic, neurological disorders, significant orthopedic injuries affecting the lower extremities, or gastrointestinal conditions that could influence visceral pain perception. Participants will also be excluded if they cannot read or comprehend the informed consent document in English.

Examiners

All assessments will be performed by trained researchers using standardized measurement techniques. The same three examiners will participate in each trial.

Examiner 1 (M.S.) will be responsible for giving informed consent, overseeing the experiment, recording any abnormal events in a notebook, and assisting with the setup and breakdown of equipment before and after testing. During the screening visit, Examiner 1 will also train participants to use the luteinizing hormone (LH) test kits and heart rate variability (HRV) test on their phones and instruct them to add the menstrual tracking calendar to their Google documents during the initial session. Examiner 1 will also be responsible for passively lowering the test leg and holding the dynamometer for strength testing.

Examiner 2 (J.F.) will be responsible for meeting the participants in the parking lot, giving them the parking pass, and escorting them into the research lab. She will also instruct the participants where they can go to void their bladder prior to testing. Examiner 2 will also be responsible for the insertion of the probe into the subject's vaginal canal and the inflation of the

barostat bulb. Examiner 2 will also relay the amount of air in the bulb when the participant experiences pain to Examiner 3.

Examiner 3 (L.D). will be responsible for placing the HRV electrodes on participants and mark in the HRV graph during each phase of the study. She will then place a mark on the greater trochanter and most posterior portion of the lateral epicondyle with an erasable marker and measure the distance between those two points. Once the participant is supine, this examiner will mark and record 5 minutes of baseline HRV data. She will then ask the participants to count their heartbeats for 3 time periods and their confidence in the count and record the results. Examiner Three will also place the biofeedback bladder under the participant's lumbar spine, monitor the dial, and instruct Examiner 1 to stop lowering the participant's test leg when the dial changes by five mmHg. Examiner 3 will then measure and record the distance from the floor to the mark on the lateral epicondyle. This examiner will also read and record the number on the dynamometer after each manual muscle test of the participant.

Screening Visit

At the initial screening visit, participants will be given informed consent and will be asked to fill out demographic questionnaires regarding medical, surgical, and gynecological history. Half of the 60 anticipated participants will fill out questionnaires regarding pain, interoceptive awareness, and quality of life at this visit, and the other half will fill out these questionnaires after the second visit to determine of questionnaires contribute to bias. Participants will be taught how to download an HRV app (Welltory) and measure their HRV via their phone camera. They will be instructed to take a one-minute measure of HRV once daily for 30 days, beginning on the first day of their period. Participants will be given an LH test kit and educated on performing LH tests daily beginning on day 10 of their menstrual cycle.

Participants will be asked to inform the research team when they detect a hormone spike via the LH kit. They will then be asked to report to the research laboratory in a sports bra without an underwire within 10 days of the spike, or during the luteal phase.

Confirming cycle status

Because the menstrual cycle phase has been shown to have an effect on pain thresholds, the menstrual cycle tracking document and LH test kits will be used to ensure participants are in the luteal phase during testing. Participants will be asked to report to the research lab within 10 days of a positive hormone spike on their LH test kit or days 15-25 of the menstrual cycle per CRAMPP studies (Hellman et al., 2020).

Ensuring Privacy and Safety

To ensure participant privacy and maintain a controlled testing environment, all assessments will be conducted in a private examination room with closed doors and covered windows to prevent external visibility. A sign will be placed outside the door to indicate that

testing is in progress, minimizing the risk of unintended entry. In the event that someone inadvertently enters, a privacy screen will be positioned between the participant and the door to prevent visual exposure and auditory privacy will be maintained by limiting conversations to necessary instructions in a low-volume, professional manner.

To safeguard the health of both examiners and participants, examiners will wear gloves and any necessary protective equipment during physical assessments of the visceral system. Some equipment, including chucks, ECG electrodes, and measuring devices, will be new and sterile for each participant. Equipment shared between participants, including gowns, linens, markers, dynamometers, and biofeedback bladder, will be sterilized between sessions to reduce the risk of cross-contamination. Hand hygiene protocols will be strictly followed, with examiners washing or sanitizing their hands before and after each session.

Experimental Protocol

The study will assess the impact of a visceral, painful stimulus on the autonomic nervous system, hip flexor mobility and dorsiflexor strength using a standardized protocol involving a barostat probe. Testing will be conducted in a temperature-controlled laboratory setting separated from the rest of the lab area by a tent with opaque walls to ensure privacy.

Preparation and Baseline Measurements

1. Participants will be met outside the building and given a parking pass to place on their dashboard. They will then be led into the testing room by the same researcher.
2. They will be instructed to void their bladder before entering the testing room.
3. Upon entry, participants will be instructed to change into a hospital gown and disposable underwear in the privacy of the testing tent.
4. Anatomical landmarks will be identified, and small circular marks will be placed on the greater trochanter and the most posterior portion of the lateral femoral epicondyle of the tested limb.
5. The distance between these markers will be measured as a calculation reference, and the distance between the marks and the floor will be used to measure hip flexor mobility.

Heart Rate Variability Assessments

6. HRV data will be recorded throughout the experiment to determine autonomic nervous system responses with Biopac MP36, three electrode system.
7. Participants will be positioned supine on an examination table, and heart rate variability (HRV) electrodes will be applied following standardized lead placement.
8. Within 15 minutes of electrode placement, a five-minute baseline HRV recording will be conducted under resting conditions per task force guidelines (Malik et al., 1996).

Interoceptive Assessments

9. Interoceptive accuracy will be assessed using a heartbeat-tracking task, with eyes open, participants will be asked to count their heartbeats for 25, 35, and 45-second intervals to quantify interoception.

10. Interoceptive confidence will be recorded using a 10 mm visual analog scale (VAS). Participants will be asked to mark the scale according to how confident they felt about their heart rate in the accuracy assessment.

Baseline Musculoskeletal Assessments

10. Hip flexor mobility will be analyzed via changes in marker distance from the floor, while dorsiflexor strength will be quantified in Newtons using the handheld dynamometer.
11. During the hip flexor measurements, a small inflatable bladder will be inflated to 40 mm Hg (Azevedo et al., 2013) and placed under the participant's lumbar spine to decrease movement from the pelvis and lumbar spine.
12. The untested leg will be positioned at 70 degrees of hip flexion to limit compensatory movement and standardize the position. The test leg will rest on a stool below the test table.
13. Hip flexor mobility will be assessed through two repeated trials using passive hip extension range-of-motion measurements. The test leg will be placed on the stool to rest between trials. Examiner 1 will passively lower the leg while Examiner 3 observes the dial on the biofeedback bladder. When the number on the biofeedback dial moves 5mm of Hg, examiner 1 will stop moving the participant's leg, and a measurement will be taken using a stadiometer (model 213, Seca, Birmingham, UK) by examiner 3. Calculations will be completed using the trigonometric technique as described by Wakefield et al. (2015) due to the improved reliability over goniometric measurements. An average of the two measurements will be taken.
14. Dorsiflexion strength will be measured via a handheld dynamometer (Hoggan Scientific Microfet 2). Dorsiflexor strength will be measured using a 'make it' technique (Spink et al., 2010) with a handheld dynamometer. In this technique, the examiner will hold the dynamometer stationary while the participant pushes against it with maximal force. Verbal encouragement will be given by examiners 1 and 3. After each trial, Examiner 1 will hold the dynamometer, allowing Examiner 3 to read and record the value. Two maximal voluntary contractions will be recorded and averaged, each separated by a one-minute rest period to mitigate fatigue.

Visceral Pain Stimulation

15. Participants will be asked to avoid taking long or short-acting over-the-counter analgesics for at least 6 hours before the assessment visit.
16. Participants will be given standardized information about the barostat probe by Examiner 2, who is credentialed by the APTA to complete internal pelvic exams.
17. To ease entry, the barostat probe will be lubricated with a water-based lubricant (Slippery Stuff, Wallace-O'Farrell, Inc., Puyallup, WA).
18. The probe will be inserted 2 inches into the vaginal canal with the participant in a supine position with lower extremities externally rotated to allow for probe insertion.
19. Following insertion, participants will resume the supine position with the test leg resting on a stool and the non-test leg at 70 deg flexion in the sagittal plane. Next, post-insertion musculoskeletal assessments will be conducted as described above:
    1. Hip flexor mobility test (two trials)
    2. Dorsiflexor strength test (two trials)

Visceral Pain Stimulation and Reassessment

20. The barostat probe will be inflated twice to allow the participant to experience the sensation.
21. Next, the barostat will be inflated to induce a visceral, painful stimulus at a pressure level individualized to the participant's tolerance. Participants will be asked to state verbally when they perceive the sensation as painful.
22. The amount of air in the probe will be noted by Examiner 2 and relayed to Examiner 3, and the bulb will be deflated.
23. Immediately following deflation, hip flexor mobility, and dorsiflexor strength will be reassessed as described above:
    1. Hip flexor mobility test (two trials)
    2. Dorsiflexor strength test (two trials)

Post-testing Instruction and Procedures

Finally, Examiner 2 will remove the probe and allow the participant to sit up on the edge of the plinth. Examiners 2 and 3 will leave the testing area, and Examiner 1 will confirm that the participant feels safe enough to stand, and she will be given a wipe to clean herself and privacy to change back into her clothes. Once fully clothed, the participant will be informed that some spotting and soreness may be present for the next two days. The participant will be reminded of the on-site healthcare options and instructed to notify her medical team if she experiences any adverse effects, as detailed in the informed consent. Any further questions from the participant will be answered by the primary investigator at this time. The participant will be thanked and escorted out of the lab.

Data analysis plan

Version 27 of IBM SPSS statistics will be used to analyze the recorded data. To determine homogeneity between groups, the baseline characteristics of the 2 groups will be analyzed using the t-test or nonparametric equivalent for continuous variables and chi-squared test for categorical variables. If participants are not able to complete all conditions, their scores will be removed via listwise deletion. Prior to data analysis, assumptions will be assessed.

The correlational analysis will be completed to determine the relationship between the variables of interest.

Regression analysis will be performed to determine the potential for variables of interest to predict outcomes in the dependent variable.

A repeated measures ANOVA (RM-ANOVA) will be used for comparing the means of the same subjects across multiple conditions (baseline, probe insertion, and probe inflation). This method can determine whether there are statistically significant changes in hip flexor mobility, dorsiflexor strength, or HRV measures over time. Assumptions include sphericity, which can be tested and corrected (e.g., using the Greenhouse–Geisser correction) if violated.

The independent variables include pain with menstruation, pain stimulus, and HRV. The dependent variables are the change in hip mobility and dorsiflexor strength following a painful stimulus.