STUDY PROTOCOL

The Effect of Menstrual Cycle on the Results of Treadmill Exercise Test with High Sensitive Cardiac Troponin Levels after Exercise in Women

Name of Study:

The Effect of Menstrual Cycle on the Results of Treadmill Exercise Test with High Sensitive Cardiac Troponin Levels after Exercise in Women

Study Design:

Observational Study Model: Case-Crossover

Time Perspective: Prospective

Biospecimen Retention: None Retained

Study coordinator and principal investigator:

Investigator: Mehmet Agirbasli MD [magirbasli]

Official Title: Medical Doctor, Professor of Cardiology

Affiliation: Istanbul Medeniyet University, Istanbul Turkey

Istanbul, Istanbul, Turkey

Co-investigators

Dr. Aysu Oktay, Affiliation: Istanbul Medeniyet University, Istanbul Turkey

Istanbul, Istanbul, Turkey

Supported by: Istanbul Medeniyet University

1. OBJECTIVE AND HYPOTHESES

Objective: To compare exercise treadmill test (ETT) results in premenopausal women at the early and late follicular phases of the menstrual cycle.

General Hypothesis:

False positive results are commonly observed in women after exercise treadmill test (ETT). The studies report severe variability in the sensitivity and specificity of ETT in detecting significant coronary artery disease (CAD). Sensitivity ranges from 23% to 100% and specificity ranges from 17% to 100% (1, 2). ETT utilizes electrocardiography (ECG) along with heart rate (HR) and blood pressure (BP) monitoring during exercise, typically involving a treadmill. The major finding of ETT is the ST segment deviation from the baseline. False positive stress ECGs are common especially in premenopausal women, limiting the specificity of the test (3-6).

Menstrual periods can cause biological variability in ETT in premenopausal women (7). The menstrual period is divided into phases: early follicular (days 1-5), late follicular (days 6-12), ovulation (days 13-15), early luteal (days 16-19), mid-luteal (days 20-23) and late luteal phases (24-28 days). Early follicular phase is characterized by low estrogen and low progesterone, late follicular phase is characterized by low progesterone and high estrogen. Mid-luteal phase is characterized by high progesterone and high estrogen levels (7-9). Early and late follicular phases of the menstrual cycle in women demonstrate nearly 4 fold differences in estrogen levels. The menstrual period phase affects thermoregulation and physical working capacity in women (9, 10). Estrogen has protective and vasodilatory effects on the cardiovascular system (11). With the discovery of high sensitive cardiac troponins (hs-cTn), minute changes of hs-cTn have become detectable (12).

The effects of menstrual periodic changes on the results of exercise treadmill test in premenopausal women have not been clearly defined. Primary purpose of the study is to investigate the biological causes of false positive test results in the treadmill exercise test in premenopausal women. Estrogen is known to have direct vasodilatory effects on coronary arteries. Early and late follicular phases of the menstrual cycle are characterized by low and high estrogen levels, respectively. The Early Follicular Phase starts on the first day of the menstrual cycle and ends when oestradiol begins to increase. It is characterised by increasing LH and FSH and constant low levels of oestradiol. The late follicular phase starts with the increase in oestradiol and ends at its preovulatory peak (7-10). False positive stress ECGs are commonly reported especially in premenopausal women, limiting the specificity of the ETT (3-5). Studies demonstrated that HR adjustment of in exercise-induced ST segment depression using the ST/HR slope or ST/HR index could improve the sensitivity and specificity of ETT (2, 13-15).

The hypothesis of the study is that ETT results and hs-cTnT release after ETT will change at the early and late follicular phases in premenstrual women.

2. STUDY ENDPOINTS

The major finding of ETT is the ST segment deviation from the baseline. The ST segments are evaluated 60-80 ms after the J point. ECG positive ETT result was defined as ≥ 1 mm (0.1 mV) horizontal or downsloping ST depression in at least 3 consecutive beats.

Chest pain or inadequate hemodynamic response during ETTs are considered clinically positive ETT result. Tests that do not meet these criteria but show ST segment changes or had low diagnostic value due to baseline ECG changes are considered indeterminate ETT results.

Automated measurements and mathematical calculations are recorded in the ETT system (General Electronic GE Healthcare T2100-ST Treadmill & CASE 6.73 Stress Test System).

ETT results are evaluated by 2 different cardiologists and categorized as positive/indeterminate/negative ETT results.

Hs-cTn is measured before and 15 minutes after ETT.

Indices of ST/HR slope and the ST/HR index were reported automatically by the General Electronic GE Healthcare T2100-ST Treadmill & CASE 6.73 Stress Test System device. The primary endpoint in the study was the ST/HR index. The secondary endpoints were ST/HR slope, maxımum ST segment depression (mm), HR and BP response to exercise, maximum exercise capacity, and the change in hotmones and hs-cTn after ETT.

Primary endpoint:

ST/HR index during ETTs at the early and late follicular phases of the menstrual cycle

(μV/beats per minute (bpm))

Secondary endpoints:

1. Change in maximal exercise capacity with exercise and menstrual cycle (METs score)
2. Maxımum ST segment depression (mm)
3. ST/HR slope (μV/beats per minute (bpm))
4. The change in high-sensitive cardiac troponin T after ETT (ng/L)
5. Hormone levels (estrogen) at the early and late follicular phases of the menstrual cycle

3. STUDY DESIGN

The study is performed in the cardiology clinics of a tertiary care training hospital. The study is approved by the local ethics committee (number 2023/0129 on 22/02/2023).

Study Population:

The study population consists of premenopausal women who applied to the outpatient clinic with chest pain between February 2023 and July 2023. Patients report that they have a regular menstrual cycle.

Written informed consent is obtained from the patients.

All patients have transthoracic echocardiography. Echocardiography demonstrates normal left ventricular systolic and diastolic function. Echocardiography does not show wall motion abnormality or significant valvular heart disease.

All patients are evaluated for cardiovascular risk. Risk factors such as hypertension, diabetes mellitus, cigarette smoking, hyperlipidemia, family history of CAD, and drug use are questioned (16). Cardiovascular risk factors are defined as follows. Hypertension is defined as a persistent elevation in office systolic BP ≥140 and/or diastolic BP ≥90 mmHg, or a 24-hr ambulatory BP monitor average of ≥130/80, or requirement of chronic use of antihypertensives. Hyperlipidemia is defined as elevated total cholesterol (>240 mg/dl), low-density lipoprotein cholesterol (LDL-C) (>160 mg /dl), triglycerides > 150 mg/dl, high-density lipoprotein cholesterol (HDL-C) (< 50 mg /dl). Cigarette smoker is defined as having smoked at least 100 cigarettes in lifetime. Type 2 diabetes mellitus is defined as fasting plasma glucose (FPG) ≥ 126 mg/dL, or 2-hour plasma glucose ≥ 200 mg/dL during a 75-g oral glucose tolerance test (OGTT), or random plasma glucose ≥ 200 mg/dL in a patient with classic symptoms of hyperglycemia, or hemoglobin A1c (HbA1c) ≥ 6.5% (48 mmol/mol). Self-reported family history of premature CAD is defined as having a first-degree relative with premature CAD (men, age <55 years; women, age <65 years).

Menstrual cycle
The history of the menstrual cycle of the patient is obtained in detail. The duration and regularity of the menstrual cycle, the amount of bleeding, and the number of days the bleeding lasted are noted for each patient. Patients with irregular menstrual cycles, breakthrough bleeding, spotting, gynecological examination, or treatment plan that would disrupt the menstrual cycle are excluded from the study.

The early and late follicular phases are determined from the history. The early follicular phase ETT is planned on the 3rd day of menstrual bleeding, while the menstrual bleeding continues. The late follicular phase ETT is planned on the 3rd day after the menstrual bleeding ends. Hormone levels are measured before each ETT to ascertain the phase of the menstrual cycle. Two ETTs are performed on each participant.

The blood sample is taken 30 minutes before the ETT to measure follicular stimulating hormone (FSH), luteinizing hormone (LH), estrogen, progesterone, and hs-cTn. After the test, the patient is allowed to rest for 15 minutes and blood is taken to check the 2nd hs-cTn after ETT.

Sampling Method: Probability Sample
Minimum Age: 20 years
Maximum Age: 55 years
Sex: Female
Gender Based: Yes
Female patients in the pre-menopausal period

The inclusion criteria are:

1. Premenopausal female patients with regular menstrual cycles,
2. ages between 20-55,
3. Patients have been seen in the cardiology clinic by a cardiologist for the symptoms of chest pain with a clinical suspicion of angina or angina equivalent.
4. A cardiologist has ordered ETT as a diagnostic test for the symptoms of chest independent of the study protocol
5. Patients can understand and sign the informed consent.

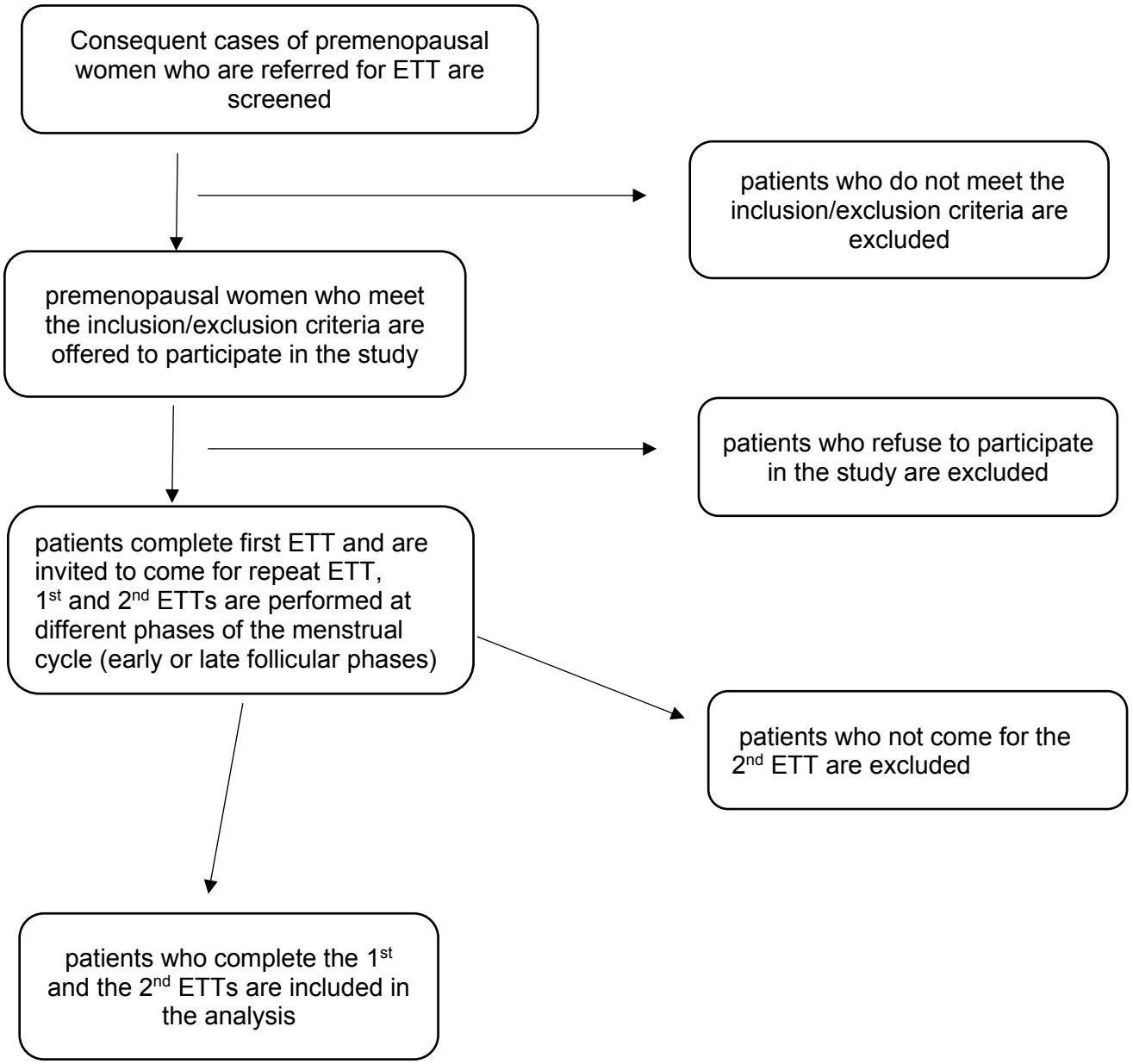
The exclusion criteria are:

1. Women with menopause or menopause-related symptoms or menstrual cycle irregularity or use of hormonal therapy or oral contraceptives, current pregnancy
2. patients with a history of CAD,
3. secondary or uncontrolled hypertension,
4. history of valvular heart disease,
5. left ventricular hypertrophy on echocardiography,
6. abnormal systolic or diastolic function or wall motion abnormality on echocardiography,
7. history of cardiac arrhythmia or heart failure, pericarditis or myocarditis
8. history of cancer, stage ≥ 3 kidney failure, liver failure, history of psychiatric illness, history of, recent infection.
9. pre-existing ECG abnormality that would confound the interpretation during the ETT (baseline ECG was considered abnormal ın the presence of the following findings: prolonged QT interval, chamber hypertrophy, alterations in the ST-T waves, any degree of heart block, T-wave abnormalities, premature ventricular contractions (PVCs), right bundle branch block (RBBB), left bundle branch block (LBBB), left or right axis deviation, or any abnormal rhythm other than normal sinus rhythm).
10. patients who cannot tolerate Bruce protocol in the first ETT
11. lack of ability or mental capacity to understand the study

4. STUDY SCHEDULE

Assessment	Screening visit-(Day 0)	Recruitment	First ETT	Phone visit on day 7 after ETT (± 2 days)	Second ETT at a different menstrual phase	Phone visit on day 7 after ETT (± 2 days)
Informed Consent Form	X	X	X		X	
Demographic characteristics	X					
Blood pressure, heart rate, and other vital signs	X	X	X	X (by phone)	X	X (by phone)
History/ Physical examination	X	X	X		X	
Hs-cTn and hormone laboratory workup			X		X	
Inclusion/exclusion criteria	X					
Pregnancy test (serum / urine)	X					

5. THE STUDY FLOW is demonstrated below.



6. REFERENCES

1. Montalescot G, Sechtem U, Achenbach S, Andreotti F, Arden C, Budaj A, et al. 2013 ESC guidelines on the management of stable coronary artery disease: the Task Force on the management of stable coronary artery disease of the European Society of Cardiology. European heart journal. 2013;34(38):2949-3003.
2. Sharma K, Kohli P, Gulati M. An update on exercise stress testing. Curr Probl Cardiol. 2012 May;37(5):177-202. doi: 10.1016/j.cpcardiol.2011.11.004. PMID: 22469057.
3. Yosefy C, Cantor A, Reisin L, Efrati S, Ilia R. The diagnostic value of QRS changes for prediction of coronary artery disease during exercise testing in women: false-positive rates. Coron Artery Dis. 2004 May;15(3):147-54.
4. Morise AP, Beto R. The specificity of exercise electrocardiography in women grouped by estrogen status. Int J Cardiol. 1997 Jun 27;60(1):55-65. doi: 10.1016/s0167-5273(97)02953-7.
5. Sun Y, Li W, Yin L, Wei L, Wang Y. Diagnostic accuracy of treadmill exercise tests among Chinese women with coronary artery diseases: A systematic review and meta-analysis. Int J Cardiol. 2017 Jan 15;227:894-900.
6. Akutsu Y, Shinozuka A, Nishimura H, Li HL, Huang TY, Yamanaka H, Takenaka H, Munechika H, Katagiri T. Significance of ST-segment morphology noted on electrocardiography during the recovery phase after exercise in patients with ischemic heart disease as analyzed with simultaneous dual-isotope single photon emission tomography. Am Heart J. 2002;144:335–342.
7. Landgren BM, Unden AL, Diczfalusy E. Hormonal profile of the cycle in 68 normally menstruating women. Acta Endocrinol. 1980;94(1):89–98.
8. Chatterton RT, Mateo ET, Hou N, et al. Characteristics of salivary profiles of oestradiol and progesterone in premenopausal women. J Endocrinol. 2005;186(1):77–84.
9. Horvath SM, Drinkwater BL. Thermoregulation and the menstrual cycle. Aviat Space Environ Med. 1982;53(8):790–4.
10. Girija B, Veeraiah S. Effect of different phases of menstrual cycle on physical working capacity in Indian population. Indian J Physiol Pharmacol. 2011;55(2):165–9.
11. Mendelsohn M, Karas RH. The protective effects of estrogen on the cardiovascular system. N. Engl. J. Med. 1999;340:1801–1811.
12. Aakre KM, Saeed N, Wu AHB, Kavsak PA. Analytical performance of cardiac troponin assays - Current status and future needs. Clin Chim Acta. 2020 Oct;509:149-155.
13. Fletcher GF, Froelicher VF, Hartley LH, Haskell WL, Pollock ML. Exercise standards. A statement for health professionals from the American Heart Association. Circulation. 1990 Dec;82(6):2286-322.
14. Okin PM, Kligfield P. Heart rate adjustment of ST segment depression and performance of the exercise electrocardiogram: a critical evaluation. J Am Coll Cardiol. 1995 Jun;25(7):1726-35. doi: 10.1016/0735-1097(95)00085-i.
15. Kardash M, Elamin MS, Mary DA, Whitaker M, Smith DR, Boyle R, Stoker JB, Linden RJ. The slope of ST segment/heart rate relationship during exercise in the prediction of severity of coronary artery disease. Eur Heart J. 1982 Oct;3(5):449-58.
16. Andersson C, Nayor M, Tsao CW, Levy D, Vasan RS. Framingham Heart Study: JACC Focus Seminar, 1/8. J Am Coll Cardiol. 2021 Jun 1;77(21):2680-2692.