**Original Research Article**

**A study of cardiovascular sympathetic function tests during different phases of menstrual cycle in young females**

Shampa Das, Sumana Panja*, Kaushik Samajdar

Department of Physiology, North Bengal Medical College, Sushrutanagar, Darjeeling, West Bengal, India

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*Correspondence:*
Dr. Sumana Panja,  
E-mail: drshampa1980@gmail.com

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**ABSTRACT**

**Background:** Menstrual cycle is a regular coordinated physiological change in non-pregnant women. The variation of hormonal concentrations during different phases of the menstrual cycle has a profound influence on autonomic and metabolic activities. The present study was designed to assess the cardiovascular sympathetic functions during different phases of menstrual cycle in normal healthy eumenorrheic females.

**Methods:** Fifty females in the age group of 18-25 years were selected for the study. Non-invasive cardiovascular sympathetic function tests were performed during different phases of the menstrual cycle using RMS Polyrite D.

**Results:** Results were analyzed using paired ‘t’ test. Resting blood pressure, blood pressure response to isometric handgrip test and cold pressor test were statistically significant higher (p-value <0.05), in the secretory phase as compared to menstrual and proliferative phase. Blood pressure response to orthostatic test was statistically significant between the proliferative and secretory phase and between menstrual and secretory phase (p-value <0.05).

**Conclusion:** Our study shows that sympathetic activity is highest during the secretory phase of the menstrual cycle and lowest in the proliferative phase as compared to the menstrual phase. This higher sympathetic activity may be correlated with higher estrogen and progesterone levels during the secretory phase of the menstrual cycle. The study also emphasizes the complex relationship between ovarian hormones and autonomic regulatory systems.

**Keyword:** Cold pressor test, Isometric handgrip exercise, Menstrual cycle, Orthostatic test, RMS Polyrite D

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**INTRODUCTION**

The menstrual cycle is a regular physiological occurrence in non-pregnant women, from puberty to menopause. It is a coordinated biological activity among hypothalamic, hypophyseal and ovarian hormones with associated structural and functional changes in the reproductive system. Changes in hormone concentrations controlled by the hypothalamo-pituitary-gonadal axis, particularly estrogen and progesterone, determine the three different phases of the normal menstrual cycle: Menstrual, Proliferative and Secretory phase.¹ The autonomic nervous system (ANS) provides the physiological background for the perceived changes. All tissues of the body except skeletal muscles are innervated by ANS. It receives information from visceral organs through the afferent arm and sends signals through the efferent arm. ANS is divided into two divisions: Sympathetic and Parasympathetic.²

Routine cardiac activity is mostly under parasympathetic control, whereas sympathetic nerves play a minor role in maintaining the cardiac function at rest. On the other hand, during physiological stress, increased sympathetic activity combined with decreased parasympathetic activity increases the rate and force of contraction of the...
heart leading to increased cardiac activity. Autonomic regulation of the heart in the normal woman differs during different phases of the menstrual cycle.\(^3\) The presence of estrogen receptors in the heart, vascular smooth muscle, and autonomic brain centers, suggest a possible involvement in the regulation of the cardiovascular system.\(^4\) Estrogen may modulate cardiovascular function through a number of mechanisms, including stress-induced activation of the hypothalamo-pituitary-adrenal and sympatho-adrenal-medullary systems.\(^5\) Endogenous progesterone is known to have a hypertensive effect, whereas estrogen promotes vasodilator effect by increased synthesis of prostacyclin and nitric oxide (NO).\(^6\) This may be responsible for the decreased sympathetic activity during proliferative phase as compared to the secretory phase. During the secretory phase, cardiac excitability may be increased by progesterone, opposing the effects of estrogen. Progesterone also exerts an inhibitory effect on the cardiovagal baroreflex responses. Thus, autonomic cardiac activity varies widely during different phases of the menstrual cycle.

A set of non-invasive, standardized, quantitative tests using cardiovascular reflexes has been widely used to assess sympathetic autonomic function. They include blood pressure changes during isometric handgrip (IHG) exercise, cold pressor test (CPT) and orthostatic test.\(^7\)

The clinical assessment of sympathetic cardiac activity in a normal menstrual cycle has great importance to understand the cardiovascular physiology in healthy women. However, limited studies were done in this field previously. So this study was conducted to assess the cardiovascular sympathetic changes during different phases of the menstrual cycle.

Objective of the study was to assess the physiological responses to non-invasive cardiovascular sympathetic functions tests during different phases of the menstrual cycle in normal healthy eumenorrheic females aged 18-25 years.

METHODS

The descriptive study was conducted on fifty normal healthy eumenorrheic females aged 18 to 25 years, after getting informed voluntary consent. Detailed history, especially menstrual history was collected and thorough general examination including height and weight measurement were done. The cycle was calculated from the date of onset of the last menstrual period and three phases are described as menstrual phase (day 1 to day 5), proliferative phase (day 6 to day 14), secretory phase (day 15 to day 28).

**Tests for cardiovascular sympathetic functions**

Cardiovascular sympathetic function tests were performed by RMS Polyrite D. The subjects were instructed to wear loose dresses. Metallic objects like rings, watch, chains, etc were removed. All the tests were conducted between 11:00 and 13:30 hours. The temperature of the examination room was between 22°C to 27°C. The subjects were also instructed not to have coffee, tea, alcohol, nicotine or cola 12 hours before the tests and avoid heavy food preceding 2 hours of testing.

**Resting blood pressure**

The subject was allowed to take rest in the supine position for 10 minutes in a quiet room to reduce the anxiety and resting blood pressure was measured.\(^8,10\)

**Blood pressure response to immediate standing from supine position (Orthostatic test)**

The subject was asked to lie down quietly for 10 minutes, baseline blood pressure was measured and then the subject was asked to stand quietly, unaided within 3 seconds and remain standing quietly for 1 minute. Blood pressure was measured immediately after standing. Difference between systolic blood pressure (SBP) at supine and erect posture was calculated. Fall in SBP less than or equals to 10 mmHg is a normal response, more than or equals to 30 mmHg is abnormal and 11 to 29 mmHg is borderline.\(^8,9\)

**Blood pressure response to isometric handgrip exercise (IHG)**

After recording baseline blood pressure in sitting position, subjects were told to hold the handgrip spring dynamometer in the right to have a full grip of it and compress the handle of the dynamometer with maximum effort for a few seconds. The whole procedure was repeated thrice with rest in between to prevent fatigue. The maximum value of the three reading was referred to as maximal voluntary contraction (MVC). Then, the subjects were asked to perform isometric handgrip exercise at 30% of MVC for 2 minutes. During the test, blood pressure was recorded from the non-exercising arm at 1 minute after the onset of handgrip and just prior to the release of the handgrip. The rise in Diastolic blood pressure (DBP) just before the release of hand grip was taken as the index of response to handgrip exercise. A rise in DBP more than or equals to 16 mmHg is normal, whereas the value of 11 to 15 mmHg is borderline and less than or equals to 10 mmHg is abnormal.\(^8,9,11\)

**Blood pressure response to cold pressor test (CPT)**

After recording baseline blood pressure in sitting position, subjects were asked to dip left hand up to the wrist in the cold water (temperature at 4° to 6° C) for 1 minute and blood pressure were recorded from the right hand at the end of 1 minute (i.e. just before the hand is taken out of cold water).
The blood pressure response to cold pressor test was taken as the increase in SBP and DBP from baseline blood pressure. The response of 15 to 20 mmHg increase in SBP and DBP by more than/equal to 10 mmHg is considered a normal response.\textsuperscript{9,10}

**RESULTS**

**Statistical analysis**

The mean, standard deviation for parameters of cardiovascular sympathetic function tests, age and Body Mass Index (BMI) were calculated. Paired t-test was applied to study the significance of differences of means for parameters at three phases of the menstrual cycle pairwise. IBM SPSS 21 and MS-Excel were used for data analysis. p-value ≤0.05 was considered statistically significant.

**Table 1: Means and standard deviations for age (in years) and BMI.**

| Variable | Mean±SD |
|----------|---------|
| Age      | 19.84±1.361 |
| BMI      | 22.88 ± 3.6 |

The mean age of the study population was 19.84±1.361 years with mean body mass index of 22.88±3.6 kg/m\(^2\). (Table 1).

**Table 2: Statistical analysis of sympathetic function tests during the menstrual and proliferative phases of the menstrual cycle.**

| Parameters (mmHg) | Menstrual Phase | Proliferative Phase | p-value |
|-------------------|-----------------|---------------------|---------|
| Resting SBP       | 112.66±3.153    | 108.76±3.761        | <0.001  |
| Resting DBP       | 73.06±4.264     | 70.08±3.416         | <0.001  |
| Rise in DBP in IHG| 18.42±3.296     | 16.60±3.024         | <0.001  |
| Rise in SBP CPT   | 19.02±2.308     | 17.26±2.717         | <0.001  |
| Rise in DBP in CPT| 11.62±2.355     | 10.44±2.296         | 0.005   |
| Fall in Postural SBP | 5.52±1.810     | 6.02±2.075          | 0.061*  |

**Table 3: Statistical analysis of sympathetic function tests during the menstrual and secretory phases of the menstrual cycle.**

| Parameters (mmHg) | Menstrual Phase | Secretory Phase | p-value |
|-------------------|-----------------|-----------------|---------|
| Resting SBP       | 112.66±3.153    | 116.84±3.548    | <0.001  |
| Resting DBP       | 73.06±4.264     | 75.88±4.251     | <0.001  |
| Rise in DBP in IHG| 18.42±3.296     | 20.08±3.20      | 0.002   |
| Rise in SBP CPT   | 19.02±2.308     | 20.04±2.424     | 0.027   |
| Rise in DBP in CPT| 11.62±2.355     | 12.64±2.328     | 0.003   |
| Fall in Postural SBP | 5.52±1.810     | 4.34±1.624      | 0.002   |

**Table 4: Statistical analysis of sympathetic function tests during the proliferative and secretory phases of the menstrual cycle.**

| Parameters (mmHg) | Proliferative Phase | Secretory Phase | p-value |
|-------------------|---------------------|-----------------|---------|
| Resting SBP       | 108.76±3.761        | 116.84±3.548    | <0.001  |
| Resting DBP       | 70.08±3.416         | 75.88±4.251     | <0.001  |
| Rise in DBP in IHG| 16.60±3.024         | 20.08±3.20      | <0.001  |
| Rise in SBP CPT   | 17.26±2.717         | 20.04±2.424     | <0.001  |
| Rise in DBP in CPT| 10.44±2.296         | 12.64±2.328     | <0.001  |
| Fall in Postural SBP | 6.02±2.075         | 4.34±1.624      | <0.001  |

Resting SBP and DBP were increased in secretory phase (116.84±3.548, 75.88±4.251) compared to menstrual (112.66±3.153, 73.06±4.264) and proliferative phase (108.76±3.761, 70.08±3.416) (p-value<0.001). (Figure 1; Table 1, 2, 3).
Blood pressure response to immediate standing (i.e. Fall in SBP) was statistically significant (p-value <0.05) between proliferative (6.02±2.075) and secretory (4.34±1.624) phase, menstrual (5.52±1.810) and secretory phase, but not significant (p-value-0.061) between menstrual and proliferative phase. (Figure 2; Table 1, 2, 3)

![Figure 1: Resting blood pressure (Mean±SD) at three phases of the menstrual cycle.](image1)

![Figure 2: Fall in systolic blood pressure (Supine to Standing) (Mean±SD) at three phases of the menstrual cycle.](image2)

![Figure 3: Rise in diastolic blood pressure in sustained handgrip test (Mean±SD) at three phases of the menstrual cycle.](image3)

![Figure 4: Rise in systolic and diastolic blood pressure in CPT (Mean±SD) at three phases of the menstrual cycle.](image4)

Blood pressure response to isometric handgrip exercise (i.e. Rise in DBP) was statistically significant during the secretory phase (20.08±3.20), compared to the menstrual phase (18.42±3.296) and proliferative phase (16.60±3.024). (Figure 3; Table 1, 2, 3)

Systolic and diastolic blood pressure rise in cold pressor test were higher in the secretory phase (20.04±2.424, 12.64±2.328) compared to menstrual (19.02±2.308, 11.62±2.355) and proliferative phase (17.26±2.717, 10.44±2.296) and the differences were statistically significant. (Figure 4; Table 1, 2, 3).

**DISCUSSION**

The level of gonadal hormones varies during different phases of the menstrual cycle, thus varying their effect on homeostatic mechanisms which regulate the cardiovascular system.12

In the present study, statistical analysis (Paired t-test) of the data of cardiovascular sympathetic function tests was done on healthy females aged 18 to 25 years, during different phases of the menstrual cycle.

Resting SBP and DBP were increased in secretory phase compared to other phases. (Figure 1; Table 1, 2, 3) The possible cause of lower resting blood pressure in proliferative phase is predominantly estrogen, which increases the density and function of presynaptic α2 adrenoreceptors, resulting in a significant decrease in norepinephrine-induced responses.13,14 Therefore, it is associated with the decreased sympathetic outflow. Estrogen also stimulates the opening of calcium-activated potassium channels by NO and cGMP dependent pathway.15 Thus estrogen relaxes vascular smooth muscle and promoting vasodilatation during the proliferative phase by stimulating the release of prostacyclin and NO. Estrogen inhibits the synthesis of vasoconstrictors like angiotensinogen II and endothelins.16,17 Therefore, the higher estrogen level in proliferative may be responsible for lower resting BP.
There was a significant rise in resting blood pressure in the secretory phase, which may be due to higher progesterone level as progesterone increases cardiac excitability by its opposing effects on estrogen in the secretory phase. Estradiol peaks in secretory phase increase the number and sensitivity of progesterone receptors, thus potentiating the action of progesterone and increased sympathetic activity. Increased level of progesterone also inhibits the release of endothelium-derived NO which is responsible for generalized vasoconstriction and increased resting blood pressure. It is also postulated that increased ovarian hormones (mainly progesterone) in the secretory phase causes increased water and salt retention along with an exaggerated increase in renin activity and aldosterone which accelerates the HR and SBP.

Our study corroborates with the study done by Kavitha C et al. They showed a significant increase in resting SBP in secretory phase compared to other phases. Rama Choudhury et al and Pechere – Bertschi et al also showed similar higher resting BP in the secretory phase. However, the study done by Mehnaz Sameera Arifuddin et al showed that though there was variation in BP between different phases of the menstrual cycle, they were not statistically significant. Dunne FP et al. showed that both SBP and DBP were higher during the menstrual phase than during other phases of the menstrual cycle.

Blood pressure response to immediate standing was statistically significant between proliferative and secretory phase, menstrual and secretory phase, but not significant between menstrual and proliferative phase. (Figure 2; Table 1, 2, 3)

On sudden change of posture from supine to standing, there is peripheral pooling of blood in the dependent parts of the body; this decreases the venous return and cardiac output resulting in a decrease in systolic blood pressure. This via sino-aortic reflex, which operates within seconds, stabilizes the blood pressure. In our study, the normal response i.e. less fall in systolic BP to immediate standing was seen in secretory phase as compared to other phases. This could be due to higher sympathetic activity in the secretory phase. This higher sympathetic activity takes care of the fall in the systolic blood pressure. A study conducted by Gridler et al found greater stokes volume responses during the secretory phase. Moreover, baroreflex control of sympathetic component increases in the secretory phase and the baroreflex regulation of autonomic function are modified by postural change during the menstrual cycle. More falls in blood pressure in the proliferative phase, compared to the menstrual phase may be due to lesser sympathetic activity in the proliferative phase.

V. Mehta – AS Chakraborty and Christina et al. showed blood pressure response to immediate standing is significant between menstrual phase and secretory phase and between proliferative and secretory phase with normal response i.e. less fall in systolic blood pressure to immediate standing was seen in secretory phase as compared to other phases. Garima Agarwal et al also showed significant blood pressure response between menstrual and secretory phase and menstrual and proliferative phase. However, a study conducted by Nir Hirshoren et al showed that orthostatic responses remain unchanged throughout the menstrual cycles and Dunne FP et al showed that blood pressure was higher at the onset of menstruation and lower during the days 17-26 of the cycle.

Blood pressure response to isometric handgrip exercise was also statistically significant during the secretory phase as compared to the menstrual phase and proliferative phase. (Figure 3; Table 1, 2, 3) During the handgrip exercise, the sympathetic nervous system is activated. Two separate theories have been suggested for this response. According to central command theory, the sympathetic nervous system is activated in parallel with α motor neurons. The exercise pressure reflex states that mechanical and chemical stimuli activate the muscle nerve fiber endings and evoke sympathetic excitation. Chemical stimulations of both myelinated and unmyelinated fibers (i.e. metaboreceptors) are likely to play a crucial role in evoking this reflex in humans. Normally during IHG, there is an increase in the concentration of metabolites like lactic acid, hydrogen ions, bradykinin and adenosine that are detected by metabolite-sensitive nerve endings within the skeletal muscle interstitium. These substances increase the discharge of group IV (metaboreceptor) afferent fibers and initiate a potent reflex that increases sympathetic activity. This leads to vasoconstriction, which contributes to the rise in BP. In animal models, estrogen promotes lipolysis and increases fatty acid availability while decreasing the rate of gluconeogenesis and sparing muscle and liver glycogen use. Estrogen up-regulates mitochondrial enzymes favoring fat oxidation. Hence estrogen might decrease the levels of lactic acid produced during exercise leading to less metaboreceptor activation.

In our study, the lower blood pressure response to sustained handgrip exercise in proliferative phase may be due to higher estrogen level in this phase. This could lead to less metaboreceptor activation during proliferative phase compared to the menstrual phase. Hence causing less sympathetic neural outflow, which intern causes a decreased blood pressure to rise in response to the handgrip test. Rinku Garg et al showed similar blood pressure response to sustained handgrip exercise. However, Christina et al showed no significant change in blood pressure response to sustained handgrip exercise throughout the menstrual cycle.

Our study showed that both systolic and diastolic blood pressure rise in response to cold pressor test was higher in the secretory phase as compared to menstrual and...
proliferative phase and the differences were statistically significant. (Figure 4; Table 1, 2, 3)

In this test, the relationship between increased muscle sympathetic nerve activity and an increase in forearm vascular resistance provoked by immersion of hand in ice-cold water is studied. This intervention produces an increase in sympathetic vasoconstriction outflow by activation of thermal and nociceptive afferents from immersed hand. Increased sympathetic activity induced by cold water stress causes norepinephrine release and elevation of blood pressure. Increased in blood pressure might also be contributed by the release of endothelins, prostaglandins and angiotensin II.34 High level of estrogen in the proliferative phase causes inhibition of production of potent vasoconstrictors like endothelins and angiotensin II, which is responsible for a lesser rise in blood pressure in cold pressor test in the proliferative phase. Estrogen causes increase density and function of presynaptic α-2 adrenergic receptors that cause a decrease in the secretion of norepinephrine.14,35 Higher estradiol levels have been shown to lower the cardiovascular response to stress, most likely through an effect on arterial wall tone and a decrease in β-receptor sensitivity to catecholamine. Therefore, the blood pressure response to the cold pressor test is lower in estrogen dominated proliferative phase as compared to the menstrual phase. On the other hand, higher resting level of plasma norepinephrine has been reported during the secretory phase of the menstrual cycle, when both concentrations of estrogen and progesterone are elevated which is responsible for norepinephrine-induced elevation of blood pressure in cold pressor test.

Authors found in our present study that systolic and diastolic blood pressure responses to cold water immersion were also greater in the secretory phase compared to menstrual and proliferative phase. Similar results were found in the study of Rinku Garg et al, where systolic and diastolic blood pressure responses to cold water were significantly higher in the secretory phase compared to proliferative and menstrual phase.11 Kavitha C. et al also showed that the mean systolic blood pressure response to cold pressor test was higher in the secretory phase compared to menstrual and proliferative phase with a statistically significant difference of systolic blood pressure between the three phases of the menstrual cycle.21 A study by Garima Agarwal et al showed that systolic and diastolic blood pressure response to cold pressor test was significantly higher in secretory phase compared to menstrual phase in contrast to a significant decrease in proliferative phase and the difference was statistically significant.18

CONCLUSION

It can be concluded from the present study that,

- The sympathetic control of the cardiovascular system is modified during the normal, regular menstrual cycle in young healthy females, with a shift towards higher sympathetic activity in the secretory phase
- This higher sympathetic activity may be due to higher estrogen and progesterone level in the secretory phase.

Our study directs the need for further research on the hormonal assay and its correlation with sympathetic activities and behavioral pattern throughout the menstrual cycle and premenstrual syndrome.

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