

Research Article**Study of Sympathetic Function Tests during Different Phases of Menstrual Cycle**Garima Agarwal^{1*}, Rakesh Kumar², Jayant Kumar³, Kuldeep Bijaraniya⁴¹Department of Physiology, Dr. S.N. Medical College, Jodhpur, Rajasthan, India²Demonstrator, Department of Physiology, BPS Govt. Medical College, Khanpur Kalan, Sonapat, India³Professor, Department of Physiology, Dr. S.N. Medical College, Jodhpur, Rajasthan, India⁴Department of Physiology, S.P. Medical College, Bikaner, Rajasthan, India***Corresponding author**

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Abstract: The fluctuation in hormonal concentrations during the menstrual cycle has profound influence on autonomic and metabolic activities. In the present study we compare the autonomic sympathetic variables to find out co- relation between menstrual phase-follicular phase and also to find out co-relation between menstrual phase-luteal phase. The present study was carried out on 150 healthy female subjects with normal menstrual cycles between the ages of 18 to 25 years, in the Department of physiology, Dr. S. N. Medical College Jodhpur. Sympathetic non-invasive autonomic function tests were performed that is cold pressure test and postural challenge test including resting blood pressure. The tests were conducted during menstrual phase, follicular phase and luteal phase of the menstrual cycle using mercury sphygmomanometer. The results were statistically analysed by applying paired “t” test. Blood pressure, orthostatic variation of blood pressure and cold pressor test were statistically significantly higher ($p < 0.05$), in luteal phase as compared to menstrual phase of menstrual cycle in contrast to the significant less ($p < 0.05$) in the follicular phase as compared to menstrual phase. The sympathetic function tests showed significant ($p < 0.01$) differences between the follicular phase-menstrual phase and Luteal phase-menstrual phase. Our study shows that sympathetic activity is highest during luteal phase and lowest in the follicular phase as compared to menstrual phase. This higher sympathetic activity may be correlated with higher oestrogen & progesterone levels during the luteal phase of the menstrual cycle. The results emerging from our study emphasize the complexity of the relationship between ovarian steroids & various hemodynamic regulatory systems.

Keywords: Sympathetic functions, Menstrual cycle, Follicular phase, Luteal phase, Menstrual phase.

INTRODUCTION

The menstrual cycle is a natural repetitive phenomenon occurring throughout the reproductive life of every woman. The biological rhythmicity of the cycle is created by the interplay among hypothalamic, hypophyseal and ovarian hormones. It is characterized by rhythmic variation in the secretion of female hormones and corresponds to changes in the reproductive organs and other physiological responses. The sex steroids are estrogen and progesterone secreted by ovaries. Cyclic fluctuations in the plasma level of these hormones contribute to the expression of the menstrual cycle.

Physiologically, Follicular phase is primarily phase of estrogen and luteal phase is primarily phase of progesterone. But in the menstrual phase there occurs sudden drop in estrogen and progesterone levels. These steroids also influence various cardio-vascular system (Blood Pressure, Heart rate, Rhythm and Vascular flow) Substrate metabolism and brain itself [1].

Cardiovascular functioning varies during the normal menstrual cycle in ways that are consistent with established cardiovascular effects of female-sex hormones [2] and cardiovascular system is governed by autonomic nervous system.

The hormonal changes to regulate the menstrual cycle were associated with physiological and psychological changes in women. Some women may experience some physical, psychological and behavioural symptoms which occur in late luteal phase of menstrual cycle as premenstrual syndrome [3]. This can be explained on the basis that-female reproductive steroids are modulators of HPA axis, which in association with ANS, form the stress system which regulates homeostatic mechanism of the body [4]. There are few studies in the cardio-respiratory functions in different phases of the menstrual cycle among the healthy Indian women. Therefore, it is worthwhile to assess autonomic functions during various phases of menstrual cycle.

MATERIAL AND METHOD

The present study was carried out on 150 normal, normotensive, nonpregnant, healthy female subjects with normal menstrual cycles between the age of 18 to 25 years, in the Department of physiology, Dr. S. N. Medical College and associated group of hospitals, Jodhpur. Which were studied for a group of Autonomic function tests, during the three phases of a single menstrual cycle. The subject selection was based on the predetermined exclusion-inclusion criteria.

Exclusion criteria

Females with history of irregular menstrual cycle for at least previous two cycles, cigarette smoking and alcohol consumption. History of OC pill consumption, long lasting backaches, heart diseases.

Prior Instructions was given to subjects to avoid heavy food preceding 2 hours of testing. No coffee, nicotine, alcohol 24 hours prior to testing. Drugs affecting cardiac autonomic functions like anti-cholinergic, over the counter cough, cold medications, sympathetic and parasympathetic drugs avoided 2 days prior. Maintain similar diet consisting of carbohydrates, fats and proteins the night prior.

All the subjects were tested under similar laboratory conditions and allowed to acclimatize themselves to the experimental and environmental conditions. The three phases of study are:

- Menstrual phase: Day 1 to day 5
- Follicular phase/Proliferative phase: Day 6 to day 14
- Luteal phase/Secretory phase : Day 15 to day 28

These tests are reliable, reproducible, simple, quick to carry out and all non-invasive. In general, blood pressure (B.P.) changes are studied to assess the integrity of the sympathetic functions. Given the complexity of the autonomic system there is no single test that precisely reflects function of a specific branch of this system. Therefore, it is not uncommon to order numerous tests based on diverse reflexes. Traditionally, batteries of autonomic tests have been introduced, with the Ewing battery being the most popular.

The following tests were performed for assessment of sympathetic activity

Resting blood pressure

Blood pressure was recorded with standard sphygmomanometer by auscultatory method. Before recording the blood pressure, subjects were allowed to rest for 5 minutes in a quiet room to reduce the anxiety. The onset of sounds (korotkoff's phase I) was taken as indicative of systolic blood pressure and disappearance of sound (korotkoff's phase V) as indicative of diastolic blood pressure. First Korotkoff sound indicated systolic blood pressure (SBP) and fifth Korotkoff sound indicated diastolic blood pressure (DBP).

Blood pressure from supine to standing position (postural challenge test)

The subject was asked to lie down quietly for 10 minutes, then the subject is asked to stand quietly, unaided within 5 seconds and remain standing quietly for 1 minute, the systolic and diastolic blood pressure was measured within 1 minute.

Cold pressure test

After recording of resting blood pressure, subject was asked to sit comfortably and immerse the hand in ice cold (4-6°C) up to wrist joint for two minutes. The after two minute subject was allowed to remove the hand. At the time of end of the test blood pressure was measured on the other arm of the subject. Maximum increases in systolic and diastolic blood pressure were determined.

Analysis of data

Analysis was done by Statistical analysis. Students't' test (two tailed dependent) has been used to find the significance. P= 0.05 was considered as statistically significant. The results so obtained were compared in both the phases as compared to menstrual phase to find out the changes in autonomic functions.

RESULTS

The autonomic function tests for sympathetic activity were compared during premenstrual, postmenstrual and during menstrual phases. A comparison was made between menstrual phase and follicular phase and similar comparison was made between menstrual phase and luteal phase.

Table 1: Comparison of autonomic functions in two different phases of menstrual cycle (The result of parameters reflecting sympathetic activity)

| Parameter (mm of Hg) | Menstrual Phase (MP) | Follicular Phase (FP) | p-value |
|----------------------|----------------------|-----------------------|---------|
| Resting SBP | 113.51±9.7 | 109.73±8.79 | p<0.01 |
| Resting DBP | 71.57±5.83 | 68.95±5.86 | p<0.01 |
| Postural SBP | 107.49±9.34 | 104.8±8.34 | p<0.01 |
| Postural DBP | 75.2±6.80 | 72.93±7.50 | p<0.01 |
| CPT SBP | 120.52±8.05 | 117.09±8.73 | p<0.01 |
| CPT DBP | 77.92±5.84 | 75.44±4.91 | p<0.01 |

Data presented in above Table 1 & 2 shows that blood pressure, orthostatic variation of blood pressure and cold pressor test were statistically significantly higher ($p < 0.05$), in luteal phase as

compared to menstrual phase of menstrual cycle in contrast to the significant less ($p < 0.05$) in the follicular phase as compared to menstrual phase.

Table 2: Comparison of autonomic functions in two different phases of menstrual cycle (The result of parameters reflecting sympathetic activity)

| Parameter (mm of Hg) | Menstrual Phase (MP) | Luteal Phase (LP) | P-value |
|----------------------|----------------------|-------------------|------------|
| Resting SBP | 113.51±9.7 | 117.53±8.94 | $p < 0.01$ |
| Resting DBP | 71.57±5.83 | 72.84±5.93 | $p > 0.05$ |
| Postural SBP | 107.49±9.34 | 110.59±10.10 | $p < 0.01$ |
| Postural DBP | 75.2±6.80 | 76.96±6.72 | $P < 0.05$ |
| CPT SBP | 120.52±8.05 | 126.04±7.96 | $p < 0.01$ |
| CPT DBP | 77.92±5.84 | 80.51±6.15 | $p < 0.01$ |

DISCUSSION

Rhythmic activities are abundant in biologic systems and the impact of these rhythms on humans is widely recognized. One such rhythm is the human menstrual cycle [5]. This is a feature which is unique to the female gender of humans and other higher apes [6]. The level of gonadal hormones varies during different phases of the menstrual cycle, thus varying their effect on homeostatic mechanisms which regulate the CVS [7].

Tests assessing autonomic function are based on evaluation of the cardiovascular reflexes triggered by performing specific provocative manoeuvres. Stimuli that raise blood pressure, such as cold pressor test, blood pressure responses to orthostatic testing are in a large part a reflection of sympathetic activity.

Resting blood pressure

In our study significant increase in the resting systolic blood pressure was observed in the luteal phase as compared to the menstrual phase in contrast to the significant decrease in the follicular phase. The possible causes of decreased sympathetic activity in the follicular phase may be due to: estrogen promotes vasodilatation by stimulating the release of prostacyclin and nitric oxide and inhibits the production of vasoconstrictors like angiotensinogen II and endothelins [8, 9]. Estrogen increases density as well as the function of presynaptic α_2 adrenoreceptors in the follicular phase that causes a significant decrease in the secretion of noradrenaline [10]. Estrogen causes smooth muscle relaxation and thus vasodilatation by stimulating the opening of calcium activated potassium channels by nitric oxide [11] and cyclic guanosine monophosphate pathway that causes vasodilatation [12]. Estradiol might also be associated with increase in acetylcholine concentration. These finding suggest that estrogen has facilitating effect on cardio-vagal function [13].

In our study significant increase in the resting systolic blood pressure was observed in the luteal phase as compared to the menstrual phase may be due to Progesterone as Progesterone may increase cardiac

excitability by its opposing effects on estrogen. Estradiol peaks during luteal phase, this increases the number and sensitivity of progesterone receptor, thus increasing action of progesterone hormone occurs during luteal phase [13]. Increase level of progesterone causes decreased release of endothelium derived nitric oxide which leads to generalized vasoconstriction and increased BP [14] as level of progesterone is significantly higher in luteal phase, causes rise in both systolic and diastolic blood pressure [15].

Higher resting levels of circulating plasma norepinephrine (a potent vasoconstrictor substance) have been reported during the secretory phase of the menstrual cycle, when both oestrogen & progesterone concentrations are elevated [16]. The plasma rennin activity and the concentrations of the fluid regulatory hormones, aldosterone and plasma norepinephrine (NE) levels increased along the luteal phase which contributes to the increased cardio-vagal baroreflex sensitivity. Physiological and psychological stress also contributes to the rise in SBP in the premenstrual phase which is also called as progesterone phase. Also the administration of exogenous progesterone and the combined oral contraceptive pills are known to induce hypertension [17]. This increased action of progesterone hormone during the luteal phase may be responsible for increased sympathetic activity.

In menstrual phase, the concentration of hormones oestrogen & progesterone is low & hence the concentrations of NO & norepinephrine may be low & therefore blood vessel diameter may not be affected & hence the resting blood pressure might have not been affected in the menstrual phase in our study.

Orthostatic variation in arterial blood pressure

In our study, blood pressure response to immediate standing (one of the sympathetic function tests) is significant between menstrual phase & follicular phase, and between menstrual phase & luteal phase. The normal response i.e. less fall in systolic blood pressure to immediate standing was seen in luteal phase as compared to menstrual phase. The normal

response to immediate standing seen during luteal phase could be due to higher sympathetic activity. This higher sympathetic activity takes care of the fall in the systolic blood pressure. A study conducted by Girdler *et al.* [18] found greater stroke volume responses during the luteal phase.

The more fall in blood pressure in follicular phase as compared to menstrual phase may be due to lesser sympathetic activity. Studies of Saeki *et al.* [19] found that Baroreflex control of the sympathetic component increases in the premenstrual phase. Thus, it was concluded that baroreflex regulation of autonomic functions is modified by postural change during the menstrual cycle. Therefore we can conclude that there is an increased postural SBP along the menstrual cycle, with more being in the luteal phase which could be attributed to the increased sympathetic activity in the luteal phase.

Cold Pressor Test

Our study showed that the systolic and diastolic blood pressure response to cold pressor test was significantly higher in the luteal phase as compared to the menstrual in contrast to the significant decrease in the follicular phases and the difference was statistically significant ($p < 0.01$).

Cold pressor test is a standard parameter to assess the activity of sympathetic nervous system [20]. Under condition of stress of either physical or psychological origin, there is activation of sympathetic nervous system. The cold water causes stimulation of cold receptors and pain receptors in the hand. The information is carried to the brain through spinothalamic pathways. The reflex involves, rise in sympathetic outflow to the vasculature and heart resulting in rise in blood pressure. Increased sympathetic activity induced by cold water stress causes norepinephrine release and elevation of blood pressure. Increase in blood pressure might also be contributed by release of endothelins, prostaglandins and angiotensin II [21]. Women pain threshold was significantly higher during the luteal phase of the menstrual cycle indicating increased levels of ovarian steroids and endorphins [22].

According to E. W. Wineman [23] the high levels of estrogen in the menstrual cycle are accompanied by decreased sympathetic nervous system activity in follicular phase. Higher estradiol levels have been shown to lower the cardiovascular responses to stress, most likely through an effect on arterial wall tone and a decrease in β -receptor sensitivity to catecholamine. Leicht *et al.* [1] suggested that, increased level of progesterone during Luteal phase inhibit the influence of estrogen on cardio-vagal activity.

Our results are in accordance with previous studies: Mehta and Chakraborty [24] and Anjali Nadir Bhat *et al.* [25] reported significantly higher SBP and increased sympathetic activity in luteal phase compared to menstrual and follicular phase. In contrast to our studies Wenner *et al.* [26] and Strauss B *et al.* [27] did not observe any demonstrable variations in heart rate and blood pressure when studied in relation to changing levels of hormones during the menstrual cycle found no differences in autonomic reactivity in different phases of menstrual cycle.

CONCLUSION

The sympathetic function tests showed significant ($p < 0.01$) differences between the follicular phase-menstrual phase and Luteal phase-menstrual phase.

Our study shows that sympathetic activity is highest during luteal phase and lowest in the follicular phase as compared to menstrual phase. This higher sympathetic activity may be correlated with higher oestrogen & progesterone levels during the luteal phase of the menstrual cycle. The results emerging from our study emphasize the complexity of the relationship between ovarian steroids & various hemodynamic regulatory systems.

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