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Research Article

Study of Parasympathetic Function Tests during Different Phases of Menstrual Cycle

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Abstract: Autonomic nerve function status may be changed during follicular and late lutealPhases of menstrual cycle due to fluctuations of serum estrogen and progesterone levels. This Alteration in autonomic nerve functions may affect cardio-vagal control. The main aim of this study is to compare the autonomic parasympathetic variables to find out co-relation between menstrual phase-follicular phases and also to find out co-relation between menstrual phase-luteal phases. Non-invasive autonomic nerve function tests like heart rate (HR) response to valsalva manoeuvre (valsalva ratio), HR response to deep breathing, HR response to standing (30th:15th ratio) were done to assess parasympathetic activity. The present study was carried out on 150 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, Jodhpur. The tests were conducted during menstrual phase, follicular phase and luteal phase of the menstrual cycle using electrocardiograph. The results were statistically analysed by applying paired "t" test. The parasympathetic activity reflects no significant ($P>0.05$) change between menstrual-follicular phases and shows no consistent pattern between menstrual-luteal phase. The data emerging from our study emphasize the complexity of the relationship between ovarian steroids & various hemodynamic regulatory systems.
Keywords: Parasympathetic nerve functions, menstrual cycle, follicular phase, luteal phase, menstrual phase.

INTRODUCTION

Menstrual cycle is a physiological cyclical occurrence in women. The biological activity of the menstrual cycle is created by the coordination among hypothalamic, hypophyseal and ovarian hormones. The menstrual cycle is governed by well-coordinated variations in the levels of ovarian estrogen and progesterone which also produces varying responses in different tissues and organs [1]. Evidences also show that various behavioural and other changes in women also occur during the menstrual cycle. Exaggerated response to hormonal changes may be responsible for different physical and psychological ramifications occurring [2].

Changes in hormone concentrations secreted by the hypothalamus-pituitary-gonadal axis, particularly estrogen and progesterone, determine the three phases that constitute the menstrual cycle: Menstrual, follicular, and luteal.

The presence of estrogens receptors in the heart, vascular smooth muscle and autonomic brain centres [3], suggest a possible involvement in the regulation of cardiovascular system. The high incidence of ischemic heart disease after menopause suggests a close association between ovarian hormone levels & the vulnerabilities in cardiovascular system [4]. In this study the co-relation between autonomic regulation of the heart and various phases of the menstrual cycle was undertaken.

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. The subject selection was based on the predetermined exclusion-inclusion criteria. The selection criteria were as follows:-

Inclusion criteria

Females with history of regular menstrual cycles with age group 18-25years.

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:-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

AUTONOMIC FUNCTIONS TESTS:

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. It is widely used in diagnosis of diabetic neuropathy and it comprises Valsalva manoeuvre, response to deep breathing, orthostatic testing.

These tests are reliable, reproducible, simple, quick to carry out and all non-invasive. In general, heart rate (H.R.) changes for the assessment of the parasympathetic functions. Heart rate changes were measured from a continuous running ECG record. All four tests for assessment of parasympathetic activity were done by using electrocardiograph machine (RMS VESTA 101). After giving rest for 5 minutes, the following parameters were recorded.

Resting heart rate

Heart rate is one of the cardiac and autonomic measurements.Heart rate was calculated using the following formula:-

$$\textbf{Heart rate}= \frac{\mathbf{1500mm}}{\mathbf{no.of\ mm\ in\ R-R\ interval}}$$

This gives the value of heart rate in beats per min.

Lying to standing test (LST/30:15 R-R ratio)

The subject was instructed to lie down comfortably, than the subject was instructed to stand within 3 seconds from lying position. The heart rate was calculated with the help of R-R interval. 30:15 R-R ratio is the longest R-R interval(Slowest HR) occurring around 30th beat after standing divided by shortest R-R interval(fastest HR) which occurs around 15th beats after standing.

LSTRatio

$$= \frac{\mathbf{longest\ R-R\ interval\ after\ standing(around\ 30th\ beat)}}{\mathbf{Shortest\ R-R\ interval\ after\ standing(around\ 15th\ beat)}}$$

30:15 ratio is considered a measure of cardiac vagal function. Normally heart rate should increase at least by 10 beats per minute in standing position.

Deep breathing test (DBT)

While recording ECG, the subject was asked to inhale deeply for 5 second followed by exhalation for 5 second at a rate of 6 breaths per minute. The ratio between longest R-R interval during expiration and shortest R-R interval during inspiration (E/I ratio) in each respiratory cycle was calculated and averaged for the total record.

E: I Ratio

$$= \frac{\mathbf{average\ of\ maximu\ R-R\ interval\ during\ expiration}}{\mathbf{average\ of\ minimum\ R-R\ interval\ during\ inspiration}}$$

Valsalva manoeuvre (VM)

The subject was asked to take deep Breath than to close both the nostrils and to blow out or to expire forcefully in rubber tube connected to sphygmomanometer and maintain air pressure at 40 mm Hg for 15 seconds. Simultaneously an ECG was recorded during VM and 30 sec after finishing it in limb II. Valsalva ratio was taken as ratio of maximum HR during the strain to the minimum HR after the strain. Alternatively, Valsalva ratio was also calculated using the following formula:-

Valsalva ratio=

$$\frac{\mathbf{Longest\ R-R\ interval\ after\ maneuveer\ during\ the\ strain}}{\mathbf{Shortest\ R-R\ interval\ during\ the\ strain}}$$

Analysis of Data

Statistical analysis: Student'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.

OBSERVATIONS & RESULT:

The autonomic function tests for parasympathetic activity were compared during premenstrual, postmenstrual and during menstrual phases. It was observed that there were no statistical significant responses to heart rate response to standing, E: I Ratio and Valsalva Ratio tests on comparison between menstrual and follicular phase of menstrual cycle, reflecting no significant change in the parasympathetic activity between menstrual and follicular phase of menstrual cycle.

Whereas the parasympathetic activity between menstrual and luteal phase show significant responses to heart rate, heart rate response to standing, but did not show significant responses for deep breathing test (E: I Ratio) and Valsalva manoeuvre, reflecting that no consistent pattern of parasympathetic activity between menstrual and luteal phase of menstrual cycle.

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

Parameter	Menstrual Phase	Follicular Phase	p-value	Significance
Resting HR	77.76±6.32	76.5±7.95	0.13*	NS
LST	1.29±0.19	1.26±0.17	0.15*	NS
EIR	1.35±0.12	1.37±0.14	0.185*	NS
VM Ratio	1.5±0.27	1.55±0.34	0.159*	NS

All values expressed as Mean ± SD; P>0.05(NS)*, P<0.05(S) **, P<0.01(HS) ***

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

Parameter	Menstrual Phase(Mp)	Luteal Phase(Lp)	P-Value	Significance
Resting HR	77.76±6.32	82.08±8.88	0.000002***	HS
LST	1.29±0.19	1.23±0.14	0.002***	HS
EIR	1.35±0.12	1.38±0.15	0.0567*	NS
VM	1.5±0.27	1.49±0.29	0.76*	NS

All values expressed as Mean ± SD; P>0.05(NS)*, P<0.05(S) **, P<0.01(HS) ***

DISCUSSION

Physiologically, Follicular phase is primarily phase of estrogen and luteal phase is primarily phase of progesterone. These steroids influence various cardio-vascular system (Blood Pressure, Heart rate, Rhythm and Vascular flow) Substrate metabolism and brain itself [8]. But in the menstrual phase there occurs sudden drop in estrogen and progesterone levels. Tests assessing autonomic function Changes in heart rate during orthostatic testing and Valsalva manoeuvre, as well as during deep breathing or diving reflex, reflect parasympathetic modulation.

Tests of autonomic cardiovascular reflexes (Parasympathetic)

Resting heart rate

In the present study, basal heart rate is increased in secretory phase as compared to menstrual phase and not significantly different in proliferative phase.

The maximum heart rate in luteal phase is may be due to progesterone as Progesterone may increase cardiac excitability, documented as an increase in the number and duration of episodes of paroxysmal supraventricular tachycardia, during the late luteal phase. A Rate pressure product (RPP) was higher in a study [5] during the luteal phase proving that progesterone may increase cardiac excitability [6]. Heart rate may also be higher in secretory phase due to higher levels of progesterone in that phase, which has thermogenic effect(increase in core body temperature of 0.3ºC-0.5ºC) [6]. Therefore we can also conclude that higher heart rate in secretory phase may also be due to the actions of progesterone. Increase in the resting heart rate points towards a lower parasympathetic activity.

Our results are in accordance with the studies of little et al [7] also showed higher heart and respiration rates during the luteal phase compared with other phases [7]. But the current result conflicts with the findings of study done by Leicht et al found that no significant differences in heart rate variability in all three phases of the menstrual cycle [8].

Lying to standing test (LST Ratio):

In the present study, we found that the mean LST ratio during menstrual phase was statistically not significant when compared to Follicular Phase. Similar comparison between menstrual and luteal phase LST ratio shows significant change Thus follicular phase on comparison with menstrual phase does not show any significant changes in LST ratio.

Changing from lying to standing position produces an integrated response of cardiovascular system which includes alteration in heart rate and blood pressure. There is a transient fall in blood pressure on standing with stimulation of carotid baroreceptor and consequent reflex tachycardia and peripheral constriction. This immediate difference in heart rate on standing could be due to withdrawal of vagal tone.

Deep breathing test for respiratory sinus arrhythmia (RSA):

In the present study we found that both follicular and luteal phases on comparison with menstrual phase do not show any significant changes in E: I ratio. Our results are similar with the studies done by: Christina has been obtained The E: I ratio was statistically not significant (p>0.05) between menstrual-luteal phase and in menstrual- follicular phase [9].

This test is based on the phenomenon of respiratory sinus arrhythmia, which is most pronounced at the respiration rate of 6 breaths per minute. Sinus arrhythmia is physiological increase in heart rate during inspiration and a decrease during expiration. Changes in heart rate with respiration provide a guide to the activity of cardiac vagal efferent control. There is a general agreement that the magnitude of this difference is a sensitive measure of vagal control of heart rate. The neuronal pathway of RSA is complex and involves baroreceptor [10]. The factors generating this respiratory sinus arrhythmia (RSA) are central spillover of impulses from respiratory centre to cardiovascular centers in the brain stem and reflexes evoked peripherally by respiratory movements [11]. The magnitude of these oscillations reflects the efferent cardiac vagal nerve activity.

Valsalva manoeuvre (Valsalva Ratio):

In the present study we found that both follicular and luteal phases on comparison with menstrual phase do not show any significant changes in VM ratio. Our results are similar with the studies done by Christina has been obtained The VM ratio was statistically not significant (p>0.05) between menstrual-luteal phase and in menstrual- follicular phase [9].

Valsalva manoeuvre evaluates function of baroreceptors. It is a voluntary forced expiration of a subject against a resistance. An increase in transthoracic pressure mechanically leads to transient increase in blood pressure (phase I), which, by activation of baroreceptors, simultaneously results in a slight bradycardia. Then, due to limited venous return and low stroke volume, blood pressure decreases with concomitant compensatory tachycardia (phase II). When the expiration is stopped (phase III), a further transient fall in blood pressure is observed because of pulmonary vasculature expansion, while heart rate increases. In phase IV, probably due to baro-receptors activation, an abrupt rise in blood pressure above the initial values with concomitant bradycardia occurs. Low-pressure mechanoreceptors are not significantly engaged during this manoeuvre. Based on changes in haemodynamic parameters, various indices can be calculated. Valsalva ratio is the most important of them and it is derived from the longest RR interval in phase IV divided by the shortest RR interval in phase II and at the very beginning of phase III.

Our results are in accordance with previous studies: Mehta. V. Chakraborthy and Wenner and Strauss B et al have studied the autonomic functions in the different phases of menstrual cycle without significant differences in parasympathetic activity [12,14,15].

Our findings are in contrast with earlier studies have shown that the ovarian hormone estrogen is known to have a multitude of effects on cardiovascular regulatory mechanism at rest and during exercise. For example, injection of estrogen into brainstem nuclei associated with the central autonomic cardiovascular control (e.g. NTS) has been demonstrated to increase vagal tone and to decrease sympathetic nerve traffic at rest and in response to hypertensive stimuli estrogen may influence the ANS function by upregulating parasympathetic activity and down regulating the sympathetic nervous system activity [13].

It is also claimed that no consistent cyclic variation of autonomic variables occurred in women and this discrepancy between results may be the cause of the difference in the variables tested and the methods employed for determining the sympathetic and parasympathetic activities [16].

So further study is required to co-relate ANS functions with hormonal changes showing significant fluctuation in reproductive steroids and also to explain the possible biomechanism underlying our finding.

CONCLUSION

On the basis of our study following conclusion was drawn: The parasympathetic activity reflects no significant change between menstrual-follicular phases and shows no consistent pattern between menstrual-luteal phase. The data emerging from our study emphasize the complexity of the relationship between ovarian steroids & various hemodynamic regulatory systems.

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