National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Association of stress with heart rate variability in different phases of the menstrual cycle

Madhurima Pullaganti[1], Siva Kumar A V[2], Maruthy K N[2], John Preetham Gurja[2], Kiran Kumar Chintala[2]

[1]MBBS Student, Narayana Medical College, Nellore, Andhra Pradesh, India, [2]Department of Physiology, Narayana Medical College, Nellore, Andhra Pradesh, India

Correspondence to: Siva Kumar A V, E-mail: reddy.sivakumar @gmail.com

Received: January 05, 2019, **Accepted:** January 22, 2019

ABSTRACT

Background: The menstrual cycle signifies a fine balance of female sexual hormones and integrity of hypothalamohypophyseal-gonadal axis, during reproductive age. Many women experience unpredictable menstruation when they encounter various kinds of stress situations that often interrupt the normal cycle. Heart rate variability is the quantitative measure of autonomic tone. **Aims and Objectives:** The present study is aimed to observe the effect of stress and autonomic balance in different menstrual cycle phases of young adolescent female medical students. **Materials and Methods:** It is a cross-sectional study where 50 young medical female students aged between 18 and 25 years were recruited and their demographic features were measured. All the study procedures except cortisol collection (early morning) were done in between 9 AM and 11 PM after 1–2 h of light breakfast. **Results:** All parameters were measured in premenstrual phase (25^{th} ± 4 days) and postmenstrual phase (10^{th} ± 5 days). Low frequency (LF) increases from 0.07 ± 0.009Hz to 0.08 ± 0.005 Hz (*P* – 0.01). However, high frequency decreases from 0.27 ± 0.02Hz to 0.26 ± 0.01Hz (*P* - 0.01). The time domain parameters SDNN 59.11 ± 24.1 increase to 74.34 ± 52.7 (*P* – 0.04). STD HR significantly reduced from 69.58 ± 12.1 to 35.36 ± 16.4 (*P* - 0.01). RMSSD increased from 47.98 ± 5.5 to 75.24 ± 14.6 (*P* – 0.02). However, NN50 reduced from 64.65 ± 12.8 to 48.52 ± 12.4 statistically significant (*P* – 0.05). However, there is no significant change in cortisol levels in both phases. **Conclusion:** The results suggest that there is a significant difference in sympathovagal balance between pre- and post-menstrual phases. LF indicates that vagal discharge is more in premenstrual phase, whereas sympathetic tone increases in postmenstrual phase.

KEY WORDS: Heart rate variability; Sympathovagal balance; Menstrual cycle; Stress; Low frequency; High frequency

INTRODUCTION

The menstrual cycle, specified by fluctuations in female sex hormones, is a fundamental physiological element that continuously influences homeostatic mechanisms in reproductive women.[1] The regular menstrual cycle denotes a woman's hormones are performing properly, and it is important to signify the body is in good health. However, a number of conditions can interfere with a woman's ability to experience a normal menstrual cycle.[2] The physiological conditions such as pregnancy, initial menarche phase, and premenopausal phase are associated with irregular menstrual cycle may be due to derangement of hypothalamo-pituitary-endocrine axis. Stress is the predominant factor that can influence regular menstrual cycle might experience unpredictable bleeding or delayed menstruation when they encounter particularly to different pressure situations such as deadlines at work or school, personal illness, or death

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2019.9.0101922012019	

of a loved one. Physical stresses such as a continuous and strenuous exercise regime or excessive weight loss often interrupt the normal duration of and pattern of menstrual cycle.[3] Any kind of stress that immediately secretes high quantity adrenal hormones, especially cortisol.[4] It has an immense effect on different nuclei of hypothalamus, accelerates cardiorespiratory activity, and declines non-essential actions like the reproductive system. The stress also interrupts hypothalamogonadal axis and fluctuations in sex hormonal levels lead to changes in the duration and behavior of menstrual cycle. The hypothalamus also creates irregular discharge through efferent autonomic nerves and it is directly proportional to the intensity of stress. In recent years, higher education has become primary cause of stress in youngsters, especially in the medical field due to substantial competition. The female medical students struggle with lot of problems such as residential lifestyle, away from parents, regular clinical duties, and handling patients create massive stress that affects their reproductive life. Apart from their immense changes in menstrual cycle, it also disorganizes regular cardiovascular parameters altered by autonomic tone. The variation in autonomic nervous system (ANS) activity influences associates with hormonal fluctuations that, in turn, lead to changes in the pattern of menstrual cycle. Conventionally, the physiological variations influenced by ANS can be evaluated by measuring heart rate, blood pressure, and traditional autonomic function tests.[5,6] In recent years, the electrophysiology has evolved remarkably. The power spectral analysis of heart rate variability (HRV) is the quantitative measure of cardiac autonomic tone which describes the sympathovagal balance.[7] The previous studies have explained changes in ANS activity in different phases of menstrual cycle. However, the association of stress with HRV was not established to the best of our knowledge. Salivary cortisol is a quantitative biomarker of stress that reflects individual nature and intensity of stress.[8] Hence, the current study was aimed to observe and establish the relationship of stress with autonomic fluctuations in different menstrual cycle phases in female adolescent medical students.

MATERIALS AND METHODS

It is an observational cross-sectional study, 50 young medical female students aged between 18 and 25 years were recruited who are studying MBBS. The subjects included in this protocol have been getting regular menstrual cycle in the past few months. The non-randomized sampling technique was followed due to sensitive information of the subjects related to menstrual cycle. This study commenced after getting ethical approval from the Institutional Ethics Committee of Narayana Medical College, Nellore, Andhra Pradesh. The ethical committee has given exemption for this project. The primary investigator recruited the subjects and implemented single blinding technique. The written informed consent was taken from each subject as per the Declaration of Helsinki (1975). All the procedures were done in the morning period, after 2 h of their light breakfast. All parameters were recorded in the Department of Physiology, Narayana Medical College, Nellore, Andhra Pradesh. However, salivary cortisol was instructed to collect in the early morning when they wake up from the bed. The menstruation is the physical sign that helps to distinguish the subjects into pre- and post-menstrual groups. Participants were casually inquired about the phase of their menstrual cycle and categorized into two groups. Premenstrual group constitutes girls yet to get menstruation (25 ± 4 days) and postmenstrual group of those who have already finished their menstruation (10 ± 5 days). They were followed, instructed, and repeated the same above procedures. Most of the participants opted out of questionnaire-based evaluation of menstrual pattern. Hence, we could not express the questionnaire data. However, the subjects agreed to participate and consented to measure the following parameters.

Anthropometric Measurements

Anthropometric measurements such as height and weight were assessed. Body mass index was calculated by dividing body weight (in kg) by height (in m) squared.

Basal Body Temperature

Before obtaining data, menstrual cycle phase was determined by basal body temperature using digital thermometer, which was correlated with the status of menstrual cycle on casual enquiry.

Salivary Cortisol

Cortisol was measured from the saliva of each participant. Saliva was collected by instructing the subject to keep a cotton plug under the tongue in the early morning before brushing. The samples were stored and run as a batch in Beckman assay (A fully automated analyzer - Clinical Laboratory Improvement Amendments) to estimate cortisol which is a biomarker of stress.

HRV

It describes the variations between consecutive heartbeats. The regulatory mechanisms of HRV originate from sympathetic and parasympathetic nervous systems. Thus, HRV can be used as a quantitative marker of autonomic nervous system. In HRV analysis, either the heart rate as a function of time or the intervals between successive QRS complexes are determined. In this study, when we talk about HRV, we actually mean the variability of RR intervals (i.e., intervals between consecutive R peaks). HRV was measured using analog electrocardiogram amplifier with analog to digital conversion using the sound card inside the computer at a sampling rate of 8000. Each data point represents 1/8th ms accuracy.[9] The RR intervals were subjected to KUBIOS 1.1 software, Finland, which was open source freeware to measure the different variables of HRV such as the time or frequency domain. The frequency domain shows the variability of the RR signal overtime by looking at the proportion of the frequencies relative to the original RR signal. Frequently used time domain parameters are mean and standard deviation (SD) of RR, NN50 (number of consecutive RR intervals that differ >50 ms), and pNN50 (proportion of NN50). Frequently used spectral measures are peak frequency and power of very low-frequency (VLF) bands, low-frequency (LF) bands, high-frequency (HF) bands, and LF/HF ratio which is often interpreted as a measure of sympathovagal balance.

Statistical Analysis

Statistical analysis was carried out using GraphPad Prism and data were represented as mean and SD. Normality of data was tested using Kolmogorov–Smirnov test. $P > 0.05$ indicated normal Gaussian distribution. As the data sets were skewed, Mann–Whitney U-test was performed. Spearman correlation was done to find out associations.

RESULTS

The present data report that the above tests were compared between the pre- and post-menstrual phases. Values are expressed as mean ± SD in Tables 1-3.

Table 1: Demographic measurements of the participants

Demographic measurements	Value in mean±SD
Age (years)	21.4±2.54
Height (cms)	159.68±4.58
Weight (kg)	59.03±6.78
BMI (kg/m^2)	23.04±1.92

BMI: Body mass index, SD: Standard deviation

Table 2: Power spectral analysis of HRV parameters measured in time and frequency domains

Power spectral HRV	Premenstrual	Postmenstrual	*P*-value
Frequency-domain analysis			
VLF Hz	0.01±0.003	0.01±0.003	0.19
LF Hz	0.08±0.005	0.07±0.009	<0.05
HF Hz	0.26±0.01	0.27±0.02	<0.05
VLF Power%	1.40±0.22	1.24±0.28	0.71
LF Power%	25.7±13.74	21.0±15.19	0.33
HF Power%	41.5±13.47	35.7±15.4	0.51
LF/HF ratio	32.3±11.79	42.8±20.11	0.15
Time domain analysis			
Mean RR (ms)	710.2±85.99	716.7±83.3	0.77
SDNN	59.11±24.1	74.34±52.7	<0.05
Mean HR	86.24±9.9	91.84±20.3	0.08
STD HR	69.58±12.1	35.36±16.4	<0.05
RMSSD	47.98±5.5	75.24±14.6	<0.05
NN50	64.65±12.8	48.52±12.4	0.05
pNN50	25.14±4.3	29.72±4.6	0.55

HRV: Heart rate variability, VLF: Very low frequency, LF: Low frequency, HF: High frequency

Table 3: Salivary cortisol and basal body temperature levels in premenstrual phase and postmenstrual phase

Parameters	Premenstrual	Postmenstrual	*P*-value
Salivary cortisol (ng/dl)	264±72.01	263.3±67.98	0.95
Body temperature (^{o})	36.78±0.67	36.79±0.59	0.89

DISCUSSION

The aim of the current study was to examine the changes in HRV and its association with stress in different phases of menstrual cycle. We explored this relation by recording the data in two phases: Before menstruation and after menstruation. The results of the current study suggest that there is a significant alteration in most of the time domain and frequency-domain parameters of HRV before and after menstruation independent of salivary cortisol.

The primary goal of this study is to find out the stress levels through salivary cortisol in pre- and post-menstrual phases. We have not found a significant difference in cortisol levels from premenstrual to postmenstrual phase. Similarly, the basal body temperature also was not changed significantly in the above-mentioned phases. The salivary cortisol levels and basal body temperatures statistically did not influence various HRV parameters. This study reports salivary cortisol levels of different phases of the menstrual cycle to the best of our knowledge. The salivary cortisol values were insignificant due to the specimen collection was done in the morning, during which basal cortisol levels were low as stress might be relieved by sleep. The various instances that control hypothalamoadrenal axis with their respective modulators, receptors, and binding proteins may affect salivary cortisol measures.[8] However, some of the studies also propose salivary alpha-amylase as a biomarker of chronic stress.[10]

We observed that there are significant differences in time domain analysis parameters such as SDNN, RMSSD, and NN50 parameters between pre- and post-menstrual phases which were largely mediated by decreased parasympathetic discharge of the autonomic nervous system. Similar results were found in the previous studies conducted in healthy women.[11]

We also found the frequency domains like low frequency indicate that predominant sympathetic discharge was decreased significantly from premenstrual phase to postmenstrual phase, whereas high frequency indicates alone vagal discharge was increased from pre- to post-menstrual phases. These results were supported by Sato *et al*. that the follicular phase was characterized by enhanced vagal activity and that the luteal phase was characterized by enhanced sympathetic activity.[12] This view was also strengthened by our findings and previous investigator's findings on resting HR, another linear indicator for assessing sympathetic activity. The observed trend toward decrease in LF may be due to the nature of the frequency band encompassing parasympathetic, sympathetic, and baroreceptor reflex activity since preliminary evidence suggest that baroreceptor reflex sensitivity is modified across the menstrual cycle.

The results regarding the influence of menstrual cycle on HRV and autonomic activity are controversial. Sato *et al*. and Yildırır *et al.* concluded that sympathetic nervous activity is predominant during the luteal phase.[13,14] Leicht *et al.* results suggested that normal variations in endogenous sex hormone levels during menstrual cycle were not significantly associated with changes in cardiac autonomic control as measured by HRV.[15] These results indicate that regulation of autonomic tone is very difficult to explain along the menstrual cycle phases.

The association of cortisol with various HRV parameters was independent as per our data. The correlation values are non-significant, may be due to other confounding factors such as day of data acquisition and intake of centrally acting beverages. This is preliminary trail tried to establish the relationship with cortisol and HRV. The present study has some limitations such as dividing the subjects into follicular, luteal, and bleeding phases of menstrual cycle were not feasible because each group would be very small without statistical power. Our study does not allow definitive conclusions to be drawn about the effects of hormonal regulation within these menstrual phases in HRV. Menstrual phase is determined by casual enquiry instead of sex hormonal levels. Hence, the menstrual phases which were considered might be not accurate. The findings of this study cannot be attributed to specific hormonal mechanisms. However, the various hormonal levels during different phases of menstrual cycle likely contribute to the observed phase variations. Further studies need to be repeated with same measurements with inclusion of more experimental days or phases and confirmation with hormonal estimations.

CONCLUSION

There is a significant difference in sympathovagal balance between pre- and post-menstrual phases. Significant LF indicates that vagal discharge is more in postmenstrual phase, whereas sympathetic tone decreased in postmenstrual phase. However, the relationship was independent of salivary cortisol levels with HRV parameters during both phases of menstrual cycle.

ACKNOWLEDGMENT

We would like to express our sincere gratitude toward the Indian Council of Medical Research (STS, Ref ID - 2018-01750) for their financial support. We would also thankful to the participants (MBBS students) and Management of Narayana Medical College and Hospital, Nellore, for their constant encouragement and support throughout the project.

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How to cite this article: Pullaganti M, Kumar AVS, Maruthy KN, Gurja JP, Chintala KK. Association of stress with heart rate variability in different phases of the menstrual cycle. Natl J Physiol Pharm Pharmacol 2019;9(3):256-260.

Source of Support: Nil, **Conflict of Interest:** None declared.