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Effect Of Endogenous Female Sex Hormone Fluctuations During Menstrual Cycle On Heart Rate Variability

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Abstract: *The aim of the present study was to investigate the influence of menstrual cycle on the cardiac autonomic function in young healthy women by power spectral analysis of heart rate variability (HRV) and to determine the correlation between estrogen, progesterone, gonadotropins and HRV in different phases of menstrual cycle. The study included 27 premenopausal women and they were assessed in both proliferative and secretory phases of menstrual cycle. Results were analyzed by using SPSS, differences between two phases compared by student's paired t test and correlation was determined by Pearson's correlation coefficient. The results showed a nonsignificant increase in high frequency (HF) normalized unit (nu) in follicular phase and low frequency (LF) nu in secretory phase. No significant correlation was found between HRV and sex hormones and gonadotropins suggesting that relationship between menstrual cycle and autonomic activity is still a matter of debate.*

Keywords: *female sex hormones, gonadotropins, HRV, menstrual cycle, power spectral analysis.*

I. Introduction

Heart is an organ under the influence of the autonomic nervous system (ANS) and one of its main characteristics is the constant modification of its rate on a beat-to-beat basis which is called Heart Rate Variability (HRV) [1,2]. Parasympathetic stimulation is associated with a decrease in heart rate and an increase in its variability whereas sympathetic stimulation is associated with an increase in heart rate and a decrease in heart rate variability. Under resting conditions, both sympathetic and parasympathetic systems are tonically active with a predominant vagal effect. A wide range of biological functions is regulated by the cyclical changes in estrogen and progesterone levels during menstrual cycle and cardiac autonomic fluctuations have been reported at various times during the menstrual cycle. However, relation between these hormones and vegetative control of the heart remains disputable due to lack of adequate studies.

Heart Rate Variability is a simple, highly sensitive, non-invasive tool to measure the periodic and non-periodic variations in beat intervals or in the instantaneous heart rate (HR) [3,4]. Power spectral density analysis provides the basic information of how power (i.e. variance) distributes as a function of frequency [2]. The High frequency (HF) band (0.15-0.4 Hz) is a marker of vagal activity and the Low frequency (LF) band (0.04-0.15 Hz) is a marker of sympatho-vagal interaction, especially sympathetic activity[2,3], whereas LF/HF ratio represents the sympatho-vagal balance[2]. Measurement of LF and HF power components is usually made in absolute values of power (ms^2), but they may also be measured in normalized units (n.u.) which represent the relative value of each power component in proportion to the total power minus the very low frequency (VLF) component [5]. Representation of LF nu and HF nu illustrates more clearly the control and balance of the two branches of the autonomic nervous system. Normalization also tends to diminish the effect of total power on values of LF and HF [2]. Nevertheless n.u. should always be quoted with absolute values of LF and HF power in order to describe in total the distribution of power in spectral components [2].

In premenopausal women, fluctuation of gonadotropins and sex hormones during normal menstrual cycle continuously affects body functions including autonomic functions [6]. The extent to which HRV is influenced by menstrual cycle is a subject of interest and more interestingly inconsistent results have been reported regarding the association between linear properties of HRV and menstrual cycle. Some investigators have found increased vagal activity during follicular phase whereas few found increased sympathetic activity during luteal phase. Few also could not find any cyclical variation of HRV along with menstrual cycle. So, this study was designed to assess whether any relationship exists between the HRV and different phases of menstrual cycle in young women.

Relationship between endogenous female sex hormones, HRV and the ANS still remains to be clarified. Blood level of reproductive hormones was not measured in most of the studies particularly serum gonadotropin (FSH & LH) levels. Moreover there is paucity in the literature regarding the association between

the HRV and endogenous level of pituitary gonadotropins and ovarian hormones. So, in this study, the relationships between the HRV and serum estrogen, progesterone as well as gonadotropin (FSH and LH) levels both in the follicular and luteal phases were evaluated. This study was designed to assess the changes in linear features of HRV during follicular and luteal phases of menstrual cycle and substantiating it with the hormonal influences in normal subjects.

We proposed two hypotheses-first, the linear changes in HRV during different phases of menstrual cycle would be related to cardiac autonomic control. Second, HRV fluctuation during menstrual cycle may result from endogenous female sex hormone and gonadotropin interaction.

II. Materials And Methods

Twenty seven healthy women aged between 17 to 33 years (21.8 ± 4.7) and Body Mass Index of 22.9 ± 6.1 kg/m^2 volunteered for this study. A detailed medical history was obtained from all participants, and they underwent a standard physiological examination. To avoid the probable influence of potential thyroid diseases on menstrual cycle, serum TSH hormone estimation was done in all cases. All subjects were euthyroid, had no heart disease, were normotensive, nondiabetic, non-pregnant and nonsmokers and they had no primary dysmenorrhea. None of them had taken any regular medication, including oral contraceptives, and they abstained from caffeine containing beverages and exercise during the study. Their menstrual cycles, including ovulation, which was performed by a urinary ovulation predictor (WH Ovultell, WHPM; El Monte, CA) for 3 months before HRV determination, were regular and ranged from 27 to 32 days (29.6 ± 2.4). All subjects gave their written informed consent before participation and all procedures were approved by the Institutional Ethics Committee.

All female subjects were studied at each of the following phases during a single menstrual cycle: the mid follicular phase (days 8-10) and the mid luteal phase (days 22-24). Both phases were further determined by serum estrogen, progesterone, LH, FSH hormones estimations in addition to a urinary ovulation predictor. To avoid potential diurnal variations, subjects were always tested at the same time of the day (between 10.00 AM to 12 noon) in the same quiet, temperature – controlled room (21-24^{0}C) after an overnight fast. All subjects were asked to be in supine position with a spontaneous breathing rate and depth with their eyes closed and in a relaxed state at least 10 min before data collection.

Digital ECG recording were taken for at least 6 min using digital polygraph 4 channel (RMS Polyrite D) with a sampling frequency of 256 Hz. From the obtained ECG amplitude values, R-R interval time series were obtained using standard algorithm. R-R interval time series data of each subject during two phases of menstrual cycle were saved, categorized and archived in specific drive destination in the PC. In the present study we utilized the Kubios HRV soft-ware package, version 2.1, released on July 2012 developed by biosignal analysis and medical imaging group, Department of Applied Physics, University of Eastern Finland (software@bsamig.uku.fi http:// kubios.uku.fi/). Every single subject specific R-R interval time series data was fed to the software package as mentioned and all the relevant parameters in frequency domain were obtained and tabulated.

After the ECG recording, 10 ml blood sample was collected and centrifuged to obtain serum. Serum ovarian hormones (17-estradiol and progesterone), Luteinizing Hormones (LH) and Follicule Stimulating Hormone (FSH), Thyroid Stimulating Hormone (TSH) were measured by Immuno-enzymatic assay using ELISA microwells (monobind).

This analysis was performed using Kubio's HRV analysis software, version 2.1, July 2012. The fast Fourier transform (FFT) spectra were squared to obtain the power spectra, from which two frequency bands [a LF band (0.04-0.15 Hz) and a HF band (0.15-0.4Hz)] were defined. Each component was expressed in power units (absolute in ms^2 and normalized in nu units); meanwhile, the ratio of LF and HF was also determined.

Statistical analysis was performed with the statistical package SPSS (IBM SPSS statistics 20). Values were expressed as means ± SD. All data were tested for normal distribution using Shapiro-Wilk test. All data were normally distributed and parametric statistical analyses were done.

Difference between the follicular phase and luteal phase for all experimental parameters, including HRV indexes and serum hormonal concentrations, were determined by the Student's paired t-test.
Correlations between serum ovarian hormone levels and HRV indexes were determined by Pearson's correlation coefficient. $P < 0.05$ was taken as statistically significant.

III. Results

Table I: Serum concentrations of ovarian hormones and gonadotropin hormones in females during both phases of menstrual cycle.

	Follicular Phase Mean ± SD N=27	Luteal Phase Mean ± SD N = 27	P Value (for difference in means using paired t test)
FSH, mIU/ml	6.191 ± 2.008	4.291 ± 2.240	0.026
LH, mIU/ml	6.067 ± 2.287	6.415 ± 4.959	0.791
FSH / LH	1.182 ± 0.476	0.937 ± 0.747	0.238
Estradiol-17, pg/ml	78.582 ± 55.315	226.602 ± 110.544	0.000
Progesterone, ng/ml	0.515 ± 0.401	17.198 ±17.368	0.000
E/P ratio	352.358 ±469.221	40.102 ±64.151	0.008

In Table I, cyclic variations in all endogenous female sex hormones are evident during the menstrual cycle. There is a significant increase in the progesterone and estrogen level in luteal phase confirming that ovulation has occurred and there is also a significant rise in estrogen in the luteal phase.
No significant differences in gonadotropins and FSH/LH ratio were observed.

Table II : Frequency domain HRV indexes in female during both phases of menstrual cycle.

	Follicular Phase Mean ± SD N = 27	Luteal Phase Mean ± SD N = 27	P Value (for difference in means using paired t test)
LF (ms^2)	2141.55± 2676.71	1601.37 ± 1400.72	0.185
LF (nu)	42.275 ± 8.128	45.415 ± 12.197	0.275
HF (ms^2)	2230.37 ± 1726.51	2389.37 ± 2316.39	0.658
HF (nu)	56.735 ± 7.867	54.985 ± 11.303	0.455
LF/HF	0.7731 ± 0.280	0.874 ± 0.454	0.357

In Table II we found that there was no statistically significant difference in the HRV indexes in the two phases of menstrual cycle studied although an increase in HF (nu) in the follicular phase compared to luteal phase of menstrual cycle and a tendency for increase in LF (nu) in luteal phase was found. Also a decrease in the absolute component of LF was found in the luteal phase while an increase in absolute component of HF was found in luteal phase though both were not statistically significant.
Correlation between reproductive hormones and different HRV indexes

Table III : Correlations among heart rate variability indexes and reproductive hormones in follicular phase of the menstrual cycle.

	Pearson's Correlation	P value
LF(ms^2) & Estradiol	r = - 0.178	0.374
LF(ms^2) & Progesterone	r = - 0.232	0.245
LF(ms^2) & FSH	r = 0.281	0.156
LF(ms^2) & LH	r = 0.239	0.231
LF(ms^2) & FSH/LH	r = - 0.152	0.451
HF(ms^2) & Estradiol	r = - 0.181	0.366
HF(ms^2) & Progesterone	r = - 0.067	0.741
HF(ms^2) & FSH	r = - 0.034	0.864
HF(ms^2) & LH	r = 0.287	0.146
HF(ms^2) & FSH/LH	r = - 0.304	0.123

Table IV: Correlations among heart rate variability indexes and reproductive hormones in luteal phase of the menstrual cycle.

	Pearson's Correlation	P value
LF(ms^2) & Estradiol	r = 0.004	0.985
LF(ms^2) & Progesterone	r = 0.135	0.501
LF(ms^2) & FSH	r = 0.071	0.725
LF(ms^2) & LH	r = - 0.035	0.862
LF(ms^2) & FSH/LH	r = 0.232	0.244
HF(ms^2) & Estradiol	r = - 0.089	0.660
HF(ms^2) & Progesterone	r = 0.296	0.134
HF(ms^2) & FSH	r = 0.073	0.716
HF(ms^2) & LH	r = - 0.106	0.598
HF(ms^2) & FSH/LH	r = 0.287	0.147

In Tables III and IV, no significant correlation was found between ovarian hormones level (17-estradiol and progesterone), as well as pituitary gonadotropin hormones level (Follicular stimulating hormone and Leutinizing hormone) with frequency domain indexes of HRV in both follicular and luteal phases.

IV. Discussion

Effect of menstrual cycle on HRV

The possible role of sex hormones on the ANS functions during the two phases of menstrual cycle has been suggested by Goldstein et al. [7] in 1983. The first clinical study supporting this hypothesis has been reported by Sato et al(1995) [8] who investigated the fluctuations of ANS activities during the follicular phase(day 7- 10 of last bleeding) and in luteal phase (3- 7 days prior to next bleeding) in 20 college students. Their results showed increased LF nu and decreased HF nu in luteal phase and obviously LF/HF ratio was significantly increased in luteal phase indicating predominant sympathetic activity during this phase. Guasti et al [9] in 1999 compared HRV of follicular phase (day 5±1) with luteal phase (day 23±3) in 13 women and showed that absolute values of LF and HF did not show any statistical significant difference but LF nu was significantly increased and HF nu was significantly decreased in luteal phase suggesting increased sympathetic activity in luteal phase. Yildirir in 2002 [10] also reported increased sympathetic/ parasympathetic ratio in luteal phase compared to follicular phase. Paula S. Mckinley et al in 2009 [11] showed a higher parasympathetic tone in mid follicular phase of the menstrual cycle compared to the mid luteal phase. These findings were corroborated by Y. Usha Rani et al in 2013 [12] concluding that there was a preponderance of parasympathetic influence in the follicular phase. But in none of these studies hormonal assays were performed.

Diametrically opposite results have been shown by other workers. Fuenmayor in 2000 [13] reported a greater parasympathetic activity during the luteal phase and he used the Valsalva index to assess heart rate response. But this study was limited in methodology as it was a crude method compared to computer based power spectral analysis of HRV. Princi et al in 2005 [14] and Chung Min Huey in 2011 [15] also demonstrated greater levels of HF during the luteal phase and Vallejo et al in 2005 [16] using 24 hour continuous ECG recordings found evidence of greater cardiac parasympathetic modulation during the luteal phase.

Leicht et al in 2002 [17] performed HRV in three phases of menstrual cycle viz. menses (day 1-5), ovulation (day11-21) and luteal (day 21—24) and they could not demonstrate any significant difference in HRV between the phases. Similarly, Nakagawa in 2006 [18] could not find any phase differences in HRV during menstrual cycle; all these results imply that the relationship between menstrual cycle and autonomic activity is still a matter of debate.

Considering the previous controversial results of the influence of menstrual cycle on linear properties of HRV, the current study showed an increased HF nu in follicular phase and higher LF nu in luteal phase with a corresponding increase in LF/HF ratio in luteal phase but none of these were found to be significant. Also interestingly the absolute power of LF component is reduced in the luteal phase. It is important to recall that during sympathetic activation, the resultant tachycardia is usually accompanied by a marked reduction in total power while the reverse occurs during vagal stimulation [2]. When the spectral components are expressed in absolute units (ms^2), the changes in total power influence LF and HF in the same direction and prevent the appreciation of the fractional distribution of the energy. Only after normalization, an increase in LF becomes evident.

Effect of reproductive hormones on HRV

The most reliable method to verify the menstrual phase is by measuring the levels of reproductive hormones in blood [19, 20], which was not done in most of the studies particularly serum gonadotropins (FSH &LH) level. Akselrod et al in 1985 [21] reported a positive relationship between estrogen and vagal activity and this was corroborated by Leicht et al in 2002 [17], who found significant correlation between estrogen levels and absolute measures of HRV at ovulation. However Leicht et al did not find any significant correlation between HRV and serum gonadotropins (FSH & LH) and estrogen and progesterone suggesting that they are not associated with modification of cardiac autonomic control. So, in this study, the relationship between the HRV and serum estrogen, progesterone as well as gonadotropins (FSH and LH) levels both in the follicular and luteal phases were evaluated. Cyclic variations in all endogenous female sex hormones were evident during the menstrual cycle with a greater estrogen level in follicular phase and significantly higher progesterone level in the luteal phase indicating that the cycles were ovulatory. There was a highly significant increase in both 17-estradiol (P value = 0.000) and Progesterone (P value = 0.000) in luteal phase compared to follicular phase. There is also a significant rise of estrogen and progesterone ratio (P value < 0.005) in luteal phase. Although there was no significant change of FSH (P value = 0.055) and LH (P value = 0.107) between the two phases but FSH/LH showed a significant change (P value <0.05). Sato et al (1995) [8] measured progesterone levels as a reliable marker of menstrual phase and observed a significant rise in this hormone in the luteal phase. In the current study, no significant correlation was found between HRV and estrogen, progesterone, FSH and LH; suggesting that these hormones were not associated with modification of cardiac autonomic control as measured by HRV. Leicht et al in 2002 [17] also could not show any significant correlation between HRV and gonadotropins but showed a positive correlation between HRV and estrogen at ovulation only.

So, in the current study we failed to demonstrate any significant influence of menstrual cycle on the linear properties of HRV, though the HF nu was increased in follicular phase and LF nu was increased in luteal phase along with increase in LF/HF ratio. Also no significant correlation between HRV and the sex hormones and gonadotropins was elucidated suggesting that these hormones were not associated with modification of cardiac autonomic control as measured by HRV.

We studied HRV at mid follicular phase and mid luteal phase during which though the estrogen level is high, there is influence of increasing levels of FSH and LH. The possible existence and extent of inhibitory influences of FSH and LH on cardiac autonomic control is presently unknown and they could account for the lack of estrogen-induced increased HRV and vagal activity during menstrual cycle as postulated by other workers. Lack of HRV fluctuation during menstrual cycle in the current study may also be attributed to the timing of the recording of HRV.

In view of the conflicting results obtained so far regarding the influence of menstrual cycle on HRV, further studies should be conducted during at least four phases of menstrual cycle viz. early follicular, mid follicular, ovulatory and mid luteal phases and correlation should be determined between the HRV indices and the sex hormones taking a larger sample size. Investigations focusing on correlation between HRV and sex hormones and gonadotropins in postmenopausal women would throw a light on the effect of sex hormones and gonadotropins on HRV. The nonlinear dynamics may also be studied for the measurement of transient changes in RR intervals.

V. Conclusion

Our findings suggest that there is no menstrual phase related differences in heart related HRV. We also conclude that though there are differences of ovarian hormone levels in different phases of menstrual cycle but there are no such significant differences of gonadotropins (FSH and LH) and no correlation exists between the different hormones and HRV in the mid follicular and mid luteal phases.

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