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COMPARISON OF CARDIAC AUTONOMIC ACTIVITY BETWEEN PRE AND POST MENOPAUSAL WOMEN USING HEART RATE VARIABILITY

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Abstract : Ageing is associated with a decline in short-term indexes of heart rate variability (HRV). But there is little evidence regarding the extent to which age-related changes in HRV depend on simultaneous changes in levels of estrogen and body composition as it occurs from pre menopausal state to postmenopausal state. The purpose of this study was (i) to compare HRV between pre and postmenopausal women, (ii) to determine whether difference in age, estrogen level and body composition could account for the difference in HRV between these two groups. HRV was assessed using spectral. analysis and estrogen level was estimated using radioimmunoassay technique. The body composition, in terms of percent fat, was assessed using measurement of skin fold thickness. Data was analyzed both before and after adjusting for age, estrogen level and body composition. It was found that the total power, high frequency (HF) and the low frequency (LF) power spectrum of HRV in absolute units were significantly lower ($P<0.001$) in postmenopausal women compared to that of premenopausal women. Postmenopausal women had significantly lower HF ($P<0.01$) and higher LF ($P<0.01$) when expressed in normalized units. The ratio of LF/HF, the index of sympathovagal balance was significantly higher ($P<0.01$) among postmenopausal women. Analysis after adjusting for age, revealed that age was one of the important confounder, responsible for the differences in all the components of power spectrum between the two study groups. Difference in estrogen level contributed for the difference in relative values of HF and LF components of HRV. Difference in body composition did not explain the difference in HRV between the groups. The study concludes that both ageing and declined estrogen levels are associated with the autonomic alterations seen among postmenopausal women.

Key words : heart rate variability pre menopausal postmenopausal ageing estrogen body composition

INTRODUCTION

Autonomic control of the heart plays an important role in cardiac mortality (1, 2). One of the main characteristics of the autonomic control to heart is the constant modification of heart rate on beat-to-beat basis. The periodic fluctuations of heart rate

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are indicative of the relative contributions of sympathetic and parasympathetic components of autonomic nervous system to the heart. Spectral analysis of HRV has shown at least two distinct regions of periodicity in heart rate. In combination with studies of various pharmacological or physiological manipulations, it has shown at least two distinct regions of periodicity in heart rate. The power at high frequency peak corresponds to respiratory sinus arrhythmia reflects mainly cardiac vagal function. The low frequency peak is influenced by baroreceptor mediated regulation of blood pressure and reflects predominantly sympathetic activity. In addition, the ratio of LF/HF has been used to reflect the sympathovagal balance. Low HRV reflects reduced parasympathetic activity or elevated sympathetic tone (3, 4, 5) and is considered an important cardiovascular risk factor (6). A reduction in HRV is associated with ageing (7), altered nutritional status (8) decreased physical activity level (9) and many other physiological conditions.

Menopause is multidimensional and influenced by many endogenous and exogenous factors mainly perceived as reproductive hormone deficiency. Their deficiency affects many metabolic and physiological functions within the women's body including cardiovascular system. Epidemiological studies have indicated that women have a lower incidence of cardiovascular disease compared to their male counterparts but this difference become decrease after menopause (10). A difference in risk for cardiovascular disease between pre-menopausal and postmenopausal women is not explained by any of the classic risk factor for heart disease. A cardio protective role for estrogen is supported by the observation that the excess risk of cardiovascular disease in women who underwent oophorectomy in young adulthood is prevented by estrogen. In addition, data shows a significant reduction in the risk of heart disease in women who take estrogen after a non-surgical menopause (11, 12).

Analysis of HRV among pre and post menopausal women can be used to evaluate the adaptations of autonomic nervous system in women related changes due to ageing, decreased beneficial effects of natural estrogen and altered body composition. Because incidence cardiovascular illness increases with ageing in women rising sharply approximately at the time of menopause, this study postulated that pre-menopausal women have greater HRV than post menopausal women. Further, difference in HRV among pre-menopausal women could be associated with age, difference in estrogen level and body fat.

In the present study our objectives were to compare heart rate variability (HRV) between premenopausal and postmenopausal women, and to determine whether difference in age, estrogen level and body composition could account for the difference in HRV between these two groups.

MATERIALS AND METHODS

Subjects

A total of 64 healthy volunteers in which, 38 premenopausal women between the age group of 20–40 years and 28 post menopausal women between the age group of 40–55 years were studied. The participants were recruited from the residents in and around the medical college. All the post menopausal women included reported that they all had menopause naturally at least two years

before. Premenopausal women had a regular menstrual cycle? The experimental procedures were performed during the follicular phase, when hormonal variations are not influenced by progesterone. After detailed enquiry of the medical history of the subjects, those with diabetes, hypertension or other cardiovascular disease, history of smoking and alcoholism were excluded. Subjects on oral contraceptive pill, hormonal replacement therapy, drugs that alter the cardiovascular functions were also excluded from the study. Informed written consent was obtained from all participants, and the experiment protocol was approved by Ethics committee of the college.

Experimental protocol

Subjects were screened after measuring height (stadiometer Nivotise Brivete Dopse, France), weight (Soehnle-Waagen GmbH and C, Murrhardt, Germany) and recording basal blood pressure and heart rate. The basal recording of blood pressure was done using sphygmomanometer by standard Riva Rocci method. The experiments were carried out in the morning in fasted state. Subjects refrained from caffeinated beverages for at least 12 hours prior to the experiments and had completed their evening meal by 9 P.M. they were also instructed to avoid strenuous physical activity from the previous evening.

All the subjects-underwent the different tests for the study in following order:

1. Blood sample collection - 5 ml of venous blood samples were drawn from ante-cubital vein from the female subjects and serum was separated. Samples were stored at –70°C until further analysis. Serum estradiol assay was performed using Enzyme-immuno-assay technique (omega diagnostic, Apha prime).
2. Anthropometric measurements: These included the measurement of skin-fold thickness at biceps, triceps, sub-scapular and supra-iliac sites. The logarithm of the sum of four skin fold was used in age and gender specific equations to obtain an estimate of body fat (13).
3. Following the anthropometric measurements, daily physical activity level was estimated using a validated physical activity questionnaire which assessed physical activity pattern over the preceding month (14). Physical activity level was calculated as 24 hour energy expenditure/basal metabolic rate (15).
4. Assessment of HRV: To quantify heart rate, the analog ECG signal was obtained using lead II to obtain a QRS complex of sufficient amplitude and stable base line. ECG signals were conveyed through an A/D converter (Biopac MP 30, Biopac system INC. Santa Barbara, CA) at a sampling frequency of 500 Hz to PC and were analyzed offline after visual checking of abnormal ECG. Heart rate variation during normal breathing for a period of 5 minutes was recorded, with subject supine, awake and resting. The data gathered was subjected to frequency domain analysis of HRV.
5. Frequency domain analysis was performed using non-parametric method of Fast Fourier Transformation. Data was edited manually for artifacts and ectopic beats. HRV software used a peak detection algorithm to find the ‘R’ wave, which was done at a resampling rate of ‘4 Hz’. A minimum of 256 data points was required to perform a spectral analysis. To attain 256 data points a duration of 5 minutes of ECG recording was required. The linear trend was

removed from each data set to avoid its contribution to low frequency power. The power frequency spectrum was subsequently quantified into standard frequency – domain measurements as defined previously (5) including total variance, HF (0.15–0.4 Hz), LF (0.04–0.15 Hz) and LF/HF.

Statistics

Statistical analysis was performed with an SPSS package (version 10.5). Normality of the distribution was assessed with the Kolmogorov-Smirnov goodness of fit test. Because of skewed distribution of absolute values of spectral powers, they were also analyzed after logarithmic transformation. Data are expressed as mean ± SEM. Data between the study groups were compared using unpaired Student t-test. Contribution of factors like age, body composition, estrogen level on the differences in HRV between two studies groups were assessed using ANCOVA. Differences were considered statistically significant at $P<0.05$.

RESULTS

Of the seventy healthy volunteers (39 premenopausal and 31 post menopausal women) originally enrolled in the study, 3 were excluded (1 premenopausal and 2 postmenopausal women) due to frequent premature ventricular beats, and inability of software to analyze the HRV.

Table I shows subject characteristics of study population. It has shown that basal systolic and diastolic blood pressure were significantly higher ($P<0.001$) in postmenopausal women than premenopausal women. The basal heart rate did not significantly differ between the study groups. Body mass index and percent fat were also significantly higher ($P<0.001$) among postmenopausal women. As expected, estrogen level in postmenopausal women was significantly lower than premenopausal women ($P<0.001$). Physical activity level did not show any difference between the study groups.

TABLE I: Subject characteristics, anthropometric measures, physical activity and estrogen level.

Parameters	*Pre-menopausal (n=39)*	*Post-menopausal (n=31)*
Age (yrs)	30.1±0.86	48.5±0.64*
Weight (kg)	53.1±0.95	56.1±0.9
Systolic blood pressure (mm of Hg)	111±6.8	118±1.73*
Diastolic blood pressure (mm of Hg)	76±0.7	81±1.2*
Basal heart rate (BPM)	64.75±1.2	66±1.3
Body mass index	21.7±0.33	23.1±0.3*
Fat percent	27.59±1.1	33.3±0.91*
Fat mass (kg)	14.28±0.9	18.2±0.02*
Fat free mass (kg)	36.92±0.57	36.5±0.78*
Physical activity level	1.65±0.02	1.69±0.02
Estrogen (pg/ml)	137 (n=18)	11.2** (n=15)

Data expressed as mean and SEM; **P<0.01; *P<0.05.

HRV parameters were analysed both before and after adjusting for age, estrogen level and body composition. Table II provides analysis of HRV expressed as total power, high frequency power spectrum, low frequency power spectrum, LF/HF ratio

TABLE II: Comparison of HRV spectral power between the study groups.

Parameters	*Pre-menopausal (n=38)*	*Post-menopausal (n=30)*
Total power (ms^2)	1524±228	478±82**
High frequency power (ms^2) (HF)	869±129	209±56**
Low frequency power (ms^2) (LF)	655±117	269±45**
High frequency (normalized)	55.46±2.5	43.81±2.43**
LF/HF	0.94±1.09	1.45±0.6*

Data expressed as mean and SEM; **P<0.01; *P<0.05.

obtained for two of the study groups. The absolute power of spectral data of HRV were analysed both prior to and following log transformation due to skewed distribution of the data. Since the results obtained were similar for both analyses, the significance levels were represented only for data prior to logarithmic transformation.

It was found that the HF, LF power spectrum of HRV in absolute units and the total power were significantly lower (P<0.001) in postmenopausal women compared to that of young women. Post menopausal women had significantly lower HF (P=0.009) and higher LF (P=0.003) when normalized for total power. The ratio of LF/HF, the index of sympathovagal balance was significantly higher (P=0.017) among postmenopausal women.

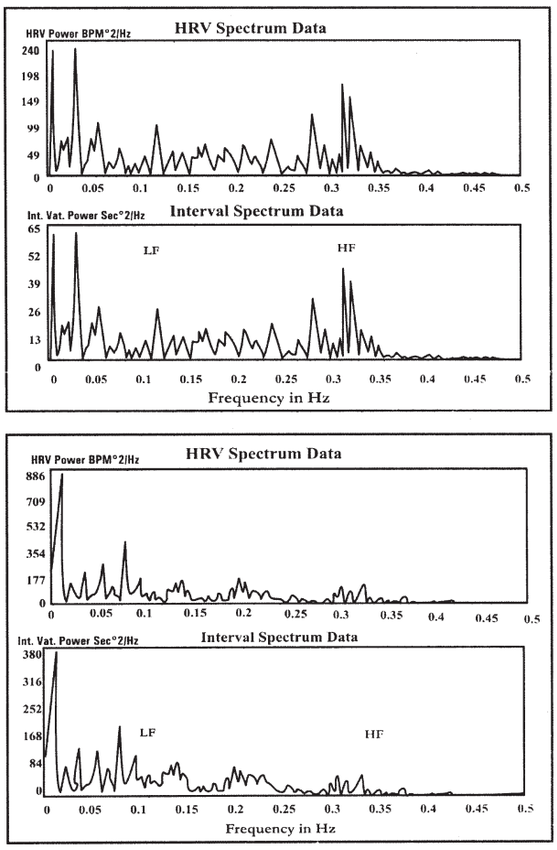

Fig. 1 : Components of HRV in premenopausal women (above) and postmenopausal women (below).

Analysis after adjusting for age using ANCOVA revealed that age was one of the important confounders responsible for the differences in all the components of power spectrum between the two study groups. Difference in estrogen level between the study groups contributed to the difference only in relative values of HF and LF components of HRV, Analysis after adjusting for percent body fat indicated “that it is not the confounding factor for difference in HRV indices between the study groups.

DISCUSSION

In testing cardiac autonomic nervous system various techniques and maneuvers have been developed to detect the integrity of the sympathetic and parasympathetic nervous system. Most of the techniques such as cold pressor test, valsalva maneuver and the tilting table tests have focused on the evoked response of autonomic nervous system (16). The evoked activities, however, might not reflect the tonic state of the body. Besides being a noninvasive study procedure, an important advantage of frequency-domain analysis of HRV is that it utilizes spontaneous fluctuation in heart rate to estimate tonic autonomic nervous functions. However, it should be noted that a controlled condition is required for spontaneous ANS functional recording. In the present study, we used spectral analysis of HRV while subjects were in supine, relaxing and rest in a quiet condition. Since, the daily physical activity level is considered as one of the potential confounders in the measurement of autonomic nerve activity (17), the study groups were controlled for the physical

activity levels in this study. Previous study (18) on comparison of HRV of pre-menopausal with postmenopausal women using time domain indexes had not controlled for the phases of menstrual cycle during subject recruitment, despite the possibility of ovarian hormonal influences on ANS function. In the present study, all pre-menopausal subjects were recruited for the study, during their follicular phase of the menstrual cycle for controlling the participants to the menstrual cycle as well as to find out the contribution estrogen for difference in HRV between the study groups. To our knowledge, this is the first report to find out the contribution of factors like age, body composition and estrogen level for difference in HRV between postmenopausal and young women.

The study indicated that there was significant difference in HRV between postmenopausal and young women. It was also found that post menopausal women had a significantly reduced overall fluctuation in autonomic input to the heart and vagal index of HRV which is reflected by lower total power and HF in absolute power in post menopausal women than young women. The higher relative power of LF and ratio of LF to HF in post menopausal women suggests that postmenopausal status is associated with shifting cardiac autonomic tone towards sympathetic dominance. Such physiological changes may be confounded by age, declined estrogen level, and increased body fat content those take place from premenopausal to postmenopausal status.

Our analysis of results after adjusting forage, indicate that age is the major determinant of HRV, accounting for the differences in all indices of HRV between the study groups. Several studies have shown reduction in autonomic modulation with ageing in both genders (19). Ageing is associated with an increased dependency on sympathetic control of cardiac responses and reduced vagal responsiveness. The blunted vagal modulation of the heart may be related to altered neural vagal discharge to sino-atrial node or to a change in the ability of the cardiac pacemaker itself. Cardiac electro physiological studies have demonstrated a progressive decline in sinoatrial conduction and sinus node recovery time with age. Studies have revealed an increase in empty Schwan cell bands, or reduced number of fibers in the vagus nerve among old subjects (20). Altered autonomic modulation with ageing also can be explained by dysfunction of baroreceptor mechanism. Increase in circulating levels of norepinephrine and thereby sympathetic over activity might account for reduced vagal efferent drive in advancing age (21).

Comparing HRV, after adjusting for estrogen level, the present study indicates that decline in estrogen level in postmenopausal women is also a confounder for difference in ratio of LF to HF between two study groups. Difference in estrogen level between the groups did not contribute for the differences in total power and absolute powers of both HF and LF. Therefore present study suggests that decline in level of estrogen from premenopausal to postmenopausal status favors the shifting of autonomic balance towards the sympathetic dominance. Premenopausal women produce other hormones in addition to estrogens. However, in this study, HRV is not influenced by progesterone since study was performed during follicular phase in premenopausal women during which progesterone concentration is only at the basal level.

Earlier studies based on the autonomic changes before and after oophorectomy in premenopausal women have shown that estrogen has a role in increasing vagal and reducing “sympathetic action (22). Similar observations were also made by studying the effect of conjugated estrogen replacement therapy in reversing the vagal deficit in postmenopausal women (23, 24). Another study which focused on the changes of autonomic functions during the menstrual cycle in premenopausal women found that parasympathetic activity is predominant in follicular phase (25). The study on cardiovascular autonomic function in postmenopausal women indicated the reduction in autonomic modulation in postmenopausal women compared to young women by estimating HRV in time domain method, where in, subject recruitment of young women was performed randomly without considering the phase of menstrual cycle (15).

Findings of our study based on frequency domain measures of HRV suggests that even in physiological doses, estrogen contributes to the alterations in sympathovagal balance. This is reflected by increased sympathovagal balance among postmenopausal women, having lower levels of estrogen. Sympathetic hyperactivity has been linked to the development of atherosclerosis, cardio-vascular hypertrophy, cardiac arrhythmia and sudden death. Elevated cardiac modulation of heart rate is associated with an increase in cardiac electrical stability and a resistance to ventricular fibrillation, thus resulting in lower risks of cardiac sudden death (26). Our findings suggest that by enhancing sympathetic dominance, decreased level of estrogen in postmenopausal women produces an unfavorable alteration in cardiac autonomic function.

There are several mechanisms through which reproductive hormonal status may influence cardiovascular autonomic reactivity. These include altering receptor sensitivity, density or neurotransmitter availability. The role of estrogen on cardiac autonomic modulation action can be explained by the effect of estrogen on enhancing the cholinergic muscaranic activity in central nervous system and such modulation at central and peripheral levels tends to suppress sympathetic but elevate parasympathetic tone (27).

Increased body fat has been associated with increased sympathetic nervous activity and decreased parasympathetic activity (28). In the present study, percent body fat was significantly higher among postmenopausal women, compared to that of young women. After adjusting for body fat, it was found that greater body fat among postmenopausal women did not contribute to the decreased HRV indices in absolute power and increased sympathovagal balance among postmenopusal women. This could be due to the recruitment of the subjects from postmenopausal groups within the normal BMI.

In conclusion, on study indicates that age is an important confounder responsible for lower HRV in postmenopausal women compared to that of young women. Further, decline in estrogen level is associated with increased sympathovagal balance in postmenopausal women.

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