

INTRODUCTION

Heart rate variability (HRV) is a measure of the extent of modulation of the cardiac sympathetic and parasympathetic tones and increased HRV is a marker of healthy cardiac autonomic activity (1). Respiration modulates the autonomic flow to the heart as evidenced by respiratory sinus arrhythmia and contributes to HRV (2). Respiratory sinus arrhythmia (3) and HRV (4, 5, 6) have been found to increase in magnitude with voluntary decrease in respiratory rate and to reach a maximum at 0.1 Hz (6 breaths/min).

A few researchers have reported that the resting respiratory rate can be modified to a lower rate by the practice of yogic breathing exercises (7, 8), but no investigation has been done to study the changes in HRV due to such a decrease in spontaneous respiratory rate. Simple, deep slow breathing exercises are often advocated as a method of relaxation. Would the daily practice of such non-yogic, deep slow breathing exercise also reduce the spontaneous breathing rate? If so, what would be its effect on the HRV? Addressing these questions, we subjected healthy volunteers to regular deep slow breathing exercise for a month and compared their respiratory rates and HRV indices before and after the breathing training.

MATERIALS AND METHODS

Participants

Subjects were enlisted from the faculty members, their spouses and from the postgraduates of the Institution, who volunteered in response to the notice

advertising for volunteers for the study. Healthy volunteers of both genders, aged 21 to 33 years, with BMI of 19 to 25 kg/m² and physical activity levels of sedentary to moderate as per a questionnaire (9) were recruited to the study. All the subjects were non-smokers and were not on any medications. Those already performing some form of yoga or breathing exercises were excluded from the study. Those with Diabetes and cardiovascular diseases were also excluded from the study. All the female subjects had regular menstrual cycles of normal duration and none of them were on oral contraceptives. The study was prior reviewed and approved by the Institutional Review Board of Christian Medical College, Vellore. Each subject gave a written informed consent prior to randomization into the intervention or control group of the study.

Experimental design

Randomised controlled trial (Registry number : CTRI/2011/091/000078)

After recruitment into the study, subjects were assigned prospectively by randomization in a 1:1 ratio into the Intervention group or the Control group of the study. The randomization was done by referring to a computer generated randomization list, prepared using block randomization method with a variable block size of 2, 4 and 6. The details of the allocation sequence were contained in a set of sealed opaque envelopes and were not known to the investigators who enrolled the participants.

A sample size of 36 subjects (18 subjects in each group) was calculated based on the results of a pilot study done on similar subjects.

A total of 59 participants were assessed for eligibility, of which 42 were recruited based on the inclusion and exclusion criteria. These 42 participants were randomized into the intervention (22 subjects) and control (20 subjects) groups. Three subjects dropped out of the study from the intervention group and the data of one subject in the intervention group and 2 subjects in the control group were not considered for analysis due to technical and procedural errors.

Baseline assessments were done on the participants at the first visit to the laboratory. The same parameters were assessed again at the second visit, one month later. Subjects in both the intervention and control group were asked to continue with their existing level of physical activity during the period of one month of the study.

Intervention: Deep slow breathing exercise

The subjects in the intervention group were asked to perform simple, deep slow breathing exercise at 6 breaths per min, daily, for half an hour, for the duration of one month of the study. They were trained to perform this breathing exercise, such that each breathing cycle consisted of 4 seconds of inspiration and 6 seconds of expiration. They were then given an audio CD with the recorded commands, prompting the timed inhalation and exhalation. They could do the half hour breathing exercise either as one sitting or as two equally divided sittings, at any convenient time of the day, either in the sitting or in the supine position adopting any convenient posture. They were given a diary to maintain a record of their everyday training. Further, continual follow up of their daily breathing practice was done through

repeated phone calls by the investigators.

The breathing exercise was given at 0.1 Hz since studies show that the modulatory effects of respiration are maximal at this breathing frequency (5, 6). Further, 0.1 Hz breathing has been reported to improve the baroreflex sensitivity and decrease the blood pressure in hypertensives (10), and improve oxygen saturation in chronic heart failure patients (8).

Expected outcomes

Change in the spontaneous resting respiratory rate was the primary outcome and changes in the Short-term HRV indices were the secondary outcome.

Acquisition of data

ECG and respiration signals were acquired using commercial leads, respiratory belt and amplifiers (BIOPAC Systems, Inc., CA 93117, USA) and stored in a personal computer. Blood pressure was recorded manually by a sphygmomanometer.

Baseline respiratory rate, heart rate, blood pressure and short-term HRV indices in the supine resting state were measured in the morning following 20 min of supine rest, 2-4 hours after a light breakfast. The subjects were asked to refrain from heavy physical activity for 24 hours and from consumption of alcohol and caffeinated beverages for 12 hours, prior to the measurements. Same parameters were assessed again in both groups at the end of one month of the study. Both the assessments were done in the same menstrual phase in all the female volunteers, except two.

HRV analysis

Published guidelines were followed for short-term HRV analysis (1). A 5-min recording of the lead II ECG, avoiding segments with ectopic beats, was subjected to short-term HRV analysis using HRV analysis software version 1.1 of the Biomedical Signal Analysis group, University of Kuopio, Finland (11). Standard time and frequency domain indices were computed.

The list of parameters studied under time domain analysis were mean (mean RR) and standard deviation (SDNN) of all the normal-to-normal RR intervals, as well as the root mean square of successive differences between adjacent RR intervals (RMSSD) and the percentage of number of RR intervals with differences >50 ms (pNN50).

RR interval data lengths of 256 seconds was cubic-spline interpolated, de-trended, Hanning windowed and fast-Fourier transformed using the averaging Welch's periodogram method to obtain the power spectral density. The frequency domain indices of low frequency (LF) and high frequency (HF) spectral powers were computed in ms^2 by integrating the power in the ranges of 0.04-0.15 Hz and 0.15-0.45 Hz respectively. The sum of the LF and HF powers was calculated as the LF+HF power. Normalized units of low (LF nu) and high (HF nu) frequency powers and the ratio of the low frequency to high frequency powers (LF/HF) were also computed under frequency domain analysis.

Statistical analysis

Study variables are presented as medians

with interquartile ranges. Wilcoxon rank sum test was used to compare the absolute change in the various parameters between the two study groups (12). Spearman's correlation coefficient test was employed to study the extent of association between variables. A P-value of less than 0.05 was considered statistically significant. All statistical analyses were done using STATA 10.0 (StataCorp, College Station, Texas, USA).

RESULTS

The demographic characteristics of the intervention group (25.6 ± 3.31 years, mean \pm SD; 7 males and 11 females; BMI of 21.9 ± 1.74 kg/m^2 , mean \pm SD) and the control group (25.5 ± 3.29 years, mean \pm SD; 11 males and 7 females; BMI of 21.7 ± 1.30 kg/m^2 , mean \pm SD) were similar.

Comparison of the absolute change [median (inter-quartile ranges)] in respiratory rate over time, showed a significant difference between the intervention and control group [intervention group -2.50 ($-4.00, -1.00$), control group 0.00 ($-1.00, 1.00$), cycles/min, $P < 0.001$] (Table I).

Similarly, comparison of the absolute changes [median (inter-quartile ranges)] in the spectral powers over time, showed a significant difference across the two groups, with respect to high frequency power [intervention group 278.50 ($17.00, 496.00$), control group -1.00 ($-341.00, 196.00$), ms^2 , $P < 0.05$] and sum of low and high frequency powers [intervention group 512.00 ($-73.00, 999.00$), control group 51.00 ($-449.00, 324.00$), ms^2 , $P < 0.05$] (Table I). The absolute changes over time in LF/HF ratio and the LF nu and HF nu were not significantly different between the two groups.

TABLE I: Comparison of absolute changes in study variables between the Control and Intervention groups.

| <i>Parameters</i> | <i>Control group n=18</i> | <i>Intervention group n=18</i> |
|--|-------------------------------|------------------------------------|
| Heart rate (bpm) | | |
| Baseline, median (IQR) | 66.87 (63.03, 70.40) | 68.79 (61.93, 72.78) |
| Median change (IQR), baseline to a month later | -0.45 (-4.83, 4.53) | -0.07 (-5.73, 3.68) |
| MAP (mmHg) | | |
| Baseline, median (IQR) | 84.33 (80.00, 90.00) | 82.00 (80, 86.67) |
| Median change (IQR), baseline to a month later | 0.667 (0.000, 6.667) | -0.667 (-6.667, 1.333)* |
| Respiratory rate (min ⁻¹) | | |
| Baseline, median (IQR) | 17.00 (16.00, 18.00) | 18.00(17.00, 20.00) |
| Median change (IQR), baseline to a month later | 0.00 (-1.00, 1.00) | -2.50 (-4.00, -1.00)*** |
| Mean RR (sec) | | |
| Baseline, median (IQR) | 0.90 (0.86, 0.96) | 0.88 (0.83,0.97) |
| Median change (IQR), baseline to a month later | 0.01 (-0.06, 0.09) | 0.01 (-0.04, 0.07) |
| SDNN (sec) | | |
| Baseline, median (IQR) | 0.06 (0.04, 0.08) | 0.06 (0.05, 0.07) |
| Median change (IQR), baseline to a month later | 0.01 (-0.01, 0.01) | 0.01 (-0.00, 0.02) |
| pNN50 (%) | | |
| Baseline, median (IQR) | 31.20(9.80, 46.80) | 30.55 (14.60, 45.20) |
| Median change (IQR), baseline to a month later | -1.50 (-17.00, 12.00) | 10.00 (-20.00, 52.00) |
| RMSSD (ms) | | |
| Baseline, median (IQR) | 56.45 (31.40, 73.10) | 56.35 (36.00, 71.20) |
| Median change (IQR), baseline to a month later | 0.15 (-10.30, 8.90) | 6.95 (-9.90, 17.10) |
| LF power (ms ²) | | |
| Baseline, median (IQR) | 343.50 (170.00, 653.00) | 352.00 (209.00, 733.00) |
| Median change (IQR), baseline to a month later | 74.50 (-110.00, 188.00) | 252.50 (5.00, 486.00) |
| HF power (ms ²) | | |
| Baseline, median (IQR) | 758.00 (184.00, 1294.00) | 505.00 (310.00, 873.00) |
| Median change (IQR), baseline to a month later | -1.00 (-341.00, 196.00) | 278.50 (17.00, 496.00)* |
| LF+HF power (ms ²) | | |
| Baseline, median (IQR) | 1398.50(362.00, 1764.00) | 1031.50 (541.00, 1351.00) |
| Median change (IQR), baseline to a month later | 51.00 (-449.00, 324.00) | 512.00 (-73.00, 999.00)* |
| LF nu | | |
| Baseline, median (IQR) | 40.10(27.50, 51.60) | 45.65 (28.30, 63.30) |
| Median change (IQR), baseline to a month later | -0.60 (-4.90, 8.00) | 2.95 (-11.30, 10.70) |
| HF nu | | |
| Baseline, median (IQR) | 59.90 (48.40, 72.50) | 54.35 (36.70, 71.70) |
| Median change (IQR), baseline to a month later | 0.60 (-8.00, 4.90) | -2.95 (-10.70, 11.30) |
| LF/HF | | |
| Baseline, median (IQR) | 0.68(0.38, 1.06) | 0.84 (0.40, 1.73) |
| Median change (IQR), baseline to a month later | -0.06 (-0.15, 0.22) | 0.01 (-0.37, 0.32) |

Values are given as median (IQR) – median (inter-quartile range). *P<0.05, ***P<0.001. Wilcoxon rank sum test.

Absolute changes of the time-domain parameters over the one month study period were not significantly different between the groups.

There was no correlation between the

observed changes in respiratory rate and LF+HF power in the intervention group (Spearman's correlation coefficient 0.27, P=0.21).

A significant difference was observed in

the absolute changes of mean arterial pressure (MAP) between the two groups [intervention group -0.67 ($-6.67, 1.33$), control group 0.67 ($0.00, 6.67$), mmHg, ($P < 0.05$)].

DISCUSSION

We compared the effect of one month of daily deep slow breathing exercise in the intervention group versus nil intervention in the control group. The outcome of the study was a significant decrease in spontaneous resting respiratory rate and an increase in the spectral indices of short-term HRV as a result of the deep breathing exercise.

It is well established that LF power reflects modulation of sympathetic tone with contribution from the parasympathetic tone, while HF power reflects the modulation of parasympathetic tone alone (13). Our findings indicate that the breathing exercise increased the modulation of the parasympathetic tone and the total resting modulation of the cardiac autonomic tones. Absence of changes in mean RR, LF/HF ratio and the LF nu and HF nu implies that the breathing exercise increased the variability without altering the proportion of the two cardiac autonomic tones and the sympatho-vagal balance.

The resting respiratory rate reduced in all the subjects of the intervention group except one, in whom the rate remained the same. In 2 subjects it decreased to 9 breaths per min and below. Two previous studies, one in heart failure patients (8) and the other in seasoned yoga practitioners (7) have reported similar findings but both involved yogic type of breathing exercises. Yogic

breathing exercises involve adapting certain bodily postures along with mental focus on the breath. Unlike these studies, we employed a simple deep slow breathing training, in which normal healthy subjects increased their tidal volumes and decreased their respiratory rates voluntarily to 6 breaths per minute, without adhering to any specific body posture or mental focus.

Previous studies have reported an automatic adjustment in tidal volumes during voluntary paced breathing at different frequencies (5). Therefore, to maintain alveolar ventilation, the tidal volume of the subjects in the intervention group would have increased during breathing training at 0.1 Hz and also when the spontaneous rates decreased after a month of training. This rationale is in keeping with the well-described inverse relationship between tidal volume and respiratory rate to maintain constant alveolar ventilation (14). It is also well known that pulmonary stretch receptor inputs and chemoreceptor inputs feed back to the medullary respiratory centres (15). It may be postulated that during the deep breathing exercise these two inputs get exaggerated due to the increased tidal volume and the larger than normal oscillations in the arterial PO_2 and PCO_2 , leading to entrainment of the central respiratory neurons and a re-setting of the respiratory rhythm. Similarly, the mechanisms producing sinus arrhythmia would have been operating at an exaggerated level during the deep breathing exercises. This would have resulted in increased modulation of the cardiac autonomic tones leading to increased HRV.

To the best of our knowledge our study

is the first to report an increase in HRV due to the practice of deep slow breathing exercises. In the studies done by Sanderson et al. (4) and Cooke et al. (5), short-term HRV parameters were found to increase when the respiratory rate was decreased voluntarily. In our study, a decrease in spontaneous breathing rate is associated with increased spectral indices of HRV. However, absence of correlation between the extent of decrease in respiratory rate and the increase in LF+HF power implies that deep breathing exercise increased the HRV spectral indices independent of the decrease in respiratory rate. The LF+HF power was chosen to study the association, since there would have been transference of power from the HF to the LF range in two of the subjects in whom the respiratory rate had decreased to 9 breaths per min (0.15 Hz) and below.

Deep slow breathing exercises are recommended as a relaxation technique and to reduce the arterial blood pressure in hypertensive patients (10, 16). In our study there was a minimal, but nevertheless significant, decrease in the MAP of the intervention group compared to the control group, attributable to the deep breathing exercise of one month. Further, the results of our study suggest that deep slow breathing exercises may also be recommended to increase the HRV in healthy subjects. Increased HRV is a marker of improved cardiac autonomic regulation and has been linked to increased longevity (17).

A limitation of our study is that though there was a significant increase in the spectral indices of HRV, a corresponding change in the time domain parameters was not observed. However, as per published

recommendations, spectral indices have better interpretable results in terms of cardiac autonomic regulation than time domain indices in short-term HRV analysis (1). Further, the increase in HRV spectral indices due to the breathing exercise observed in our study was not of large magnitude. This is probably because of the very high baseline levels of HRV in the young and healthy subjects recruited in our study.

Published literature reports that HRV is reduced with ageing (18), stress (19, 20) and in certain disease conditions (1). Would the practice of deep slow breathing exercise in such situations help to increase the HRV and improve the cardiac autonomic status associated with the condition? Adams et al. (21) have reported that the daily practice of breathing at 6 breaths/min did not alter the HRV parameters in post myocardial infarction or coronary artery bypass graft surgery patients. However, the control and intervention group of patients in their study were in an ongoing cardiac rehabilitation programme, which would have altered the HRV in both the groups. Further, 77% of the patients in their study were on heart rate lowering medications which could have prevented the manifestation of the beneficial effects of breathing exercises. The authors themselves have stated these limitations of their study.

Our study confirms that simple deep slow breathing exercise, without any associated yogic practices, decreases spontaneous respiratory rate and increases the spectral indices of short-term HRV. Further studies on an elderly population or on individuals known to have reduced HRV due to stress or disease are warranted to explore the

effects of deep slow breathing exercises in improving cardiac autonomic control in such situations.

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