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Effect of 40 continuous connected breathing on electroencephalogram and heart rate variability of healthy volunteers

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ABSTRACT

Objectives: Continuous connected breathing (CCB) is a high-frequency breathing technique without breath retention. This study examines changes in electroencephalogram (EEG) band power and heart rate variability (HRV) before, during and after breathwork intervention of 3 rounds of 40 CCB versus normal breathing in healthy volunteers.

Materials and Methods: Thirty-three healthy volunteers, aged between 19 and 23 years, participated in the study (n = 15; female = 7; mean age = 21.5 years) group, while the control group consisted of 18 individuals (n = 18; male = 11; mean age = 19.6 years). After acquiring baseline measures, the study group engaged in 40 CCB practice online for 10 days, while the control group continued with their usual activities. EEG and HRV signals were recorded for both groups after the 10 days. Post-intervention, the study group practised 40 CCB breathwork, while the control group breathed normally during signal acquisition. EEG was recorded for 1-min durations at five different time events (1st, 4th, 5th, 7th and 10th min) according to the protocol. HRV was continuously recorded for the entire 10 min, and 1-min readings corresponding to the EEG recording time intervals were analysed. EEG signals were acquired using an Enobio-8 cap with dry electrodes in pre-frontal (FP1 and FP2), temporal (T7 and T8), central (C3 and C4) and occipital (O1 and O2) regions. HRV data were acquired from Polar H7/H10 chest strap sensors through Bluetooth using Elite HRV app on android phone and data were later processed with Kubios software. EEG signals were processed using MATLAB software to extract EEG power for delta (0–3.5Hz), theta (4–7 Hz), alpha (8–13 Hz), beta (14–29 Hz) and gamma frequencies (30–50 Hz). The EEG power and HRV signals were later arranged as before-after and during time events and statistically analysed for within and between-group changes using t-tests for significance of means.

Results: There was an overall quantitative change in EEG band power of −2 units within the study group and −0.03 within the control group. A significant decrease was found in overall delta (−2.27), theta (−1.7), alpha (−1.8) and beta (−2.27) brain waves in pre-frontal (FP1, FP8), temporal (T7, T8) and occipital (O1, O2) regions after breathwork compared to before within the study group. A significant increase was found in beta (+2.8) band power in the control group with a higher increase in the left compared to the right hemisphere. Bilateral average change ranged from −1.6 to −2.6 in the study and −0.1 to +4.9 in the control group. HRV parameters were reduced after intervention in both groups but without significance. The parameters improved after the intervention compared to during within the study group, though not significant.

Conclusion: 10 days of practice of 40 CCB breathworks (3 rounds) for 5 min/day may reduce EEG power in delta, theta, alpha and beta waves. It may help to maintain autonomic balance with an increase of HRV in the recovery phase after an initial decrease during breathwork. Further studies are recommended to test the consistency of outcomes.

Keywords: Electroencephalogram power, Brain waves, Heart rate variability, 40 CCB, Breathworks

INTRODUCTION

Breathworks with volitionally controlled accelerated breath patterns have origins in shamanic and yogic traditions for over 10,000 years. Amongst them, Kapalabhati and Bhastrika are the few most ancient practices documented in yoga traditions and are generally practised either at a slow/fast pace with/without short breaks, though literature is ambiguous about practice mechanisms such as the rate and intensity. Other cyclical breathing patterns such as holotropic breathwork (HB), rebirthing breathwork (RB) and holorenic breathwork (HrnB) include continuous connected breathing (CCB) without breath retention as an element of their practice. The Sudarshan Kriya Yoga (SKY) includes slow and fast cyclical breathing patterns ranging from 8-14 respiratory cycles/min to 40–50/60–100 cycles/min with forceful exhales, and rapid inhales; the fast pace with the approximate rate of 30 breaths/min.[1,2] HB therapy combines rapid and deep breathing[3] with music and other elements. HrnB[4] usually lasts 2–3 hours and is based on kapalabhati with a pace of 140–160 breaths/min with music and bodywork. Breathworks with CCB may induce altered states of consciousness and also a ‘no experience’ state, which may be associated with cessation of thoughts with suspension of breath without fatigue or drowsiness. It can also improve anxiety and stress and increase heart rate variability (HRV) and cognitive functions.[4-6]

The common brain waveforms observed in electroencephalogram (EEG) studies are delta (0.5–4Hz), theta (4–7 Hz), alpha (8–12 Hz), beta (13–30 Hz) and gamma (>30 Hz) as in the current study. Delta activity in wakeful states is associated with concentration-related activities (mental calculations, memory and learning) and is higher during cognitively difficult tasks compared to the easier ones.[7] The theta waves are stronger during internal focus activities, mostly frontal regions. The alpha waves are found in the occipital region in a normal wakeful state and generally identify with a relaxed mental state. The beta waves are frequently observed in frontal and central regions and are associated with high concentration and alertness during normal drowsiness or as a result of sedative medications.[8] The gamma waves are observed in all parts of the brain and are associated with enhanced attention and neural activation, helping in the performance of higher mental and perceptive tasks.[9] The theta, alpha, beta and gamma EEG power reduces during inspiration of faster breathing and increases during normal/slow breathing.[10] The temporal delta increases during slow or spontaneous inspiration, while the parietal delta and total power in the frontal decrease during normal inspiration.[11] Respiration rates, cardio and brain activities are interdependent with mutual dynamic influences helping one to adapt to real-time situations. HRV, or change in time intervals between the heartbeats, is hence an indicator of dynamic interactions or the self-regulatory capacity often influenced by autonomic neural and cardiorespiratory activities.[12-14]

The root mean square of successive RR interval differences (RMSSD) in the time domain is associated with parasympathetic output, and the standard deviation of normal-to-normal RR intervals (SDNN) is a marker for both sympathetic and parasympathetic activities. The frequency domain is vagally mediated with a high frequency (HF) (0.15–0.4 Hz) component associated with parasympathetic activity and corresponds to heart rate (HR) changes influenced by respiration. The low frequency (LF) (0.04–0.15 Hz) is also associated with changes in vagally mediated baroflex activity. High frequency breathing (HFB) is similar to regular exercise that may boost stress tolerance and improve physiological and psychological well-being.[13-15] The alveolar carbon dioxide levels of a 2–5 min of kapalabhati practice at 120 breaths/min are identical to normal resting.[16,17] Kapalabhati increases breath-holding,[18] causes significant changes in alpha, theta and beta wave activities and induces subjective relaxation.[19] SKY practised for 35–40 min, increases alpha wave activity, synchronises left and right brain activities and increases EEG spectral frequencies of frontal, central, parietal, temporal and occipital brain regions.[19,20]

However, since limited studies have examined the effect of short-duration HFBs, the current study aims to examine the effect of 40 CCB (>0.5 Hz) on EEG and HRV.

MATERIALS AND METHODS

A total of thirty-three healthy individuals in the age group 19–23 years were recruited from colleges through online advertisements as per inclusion criteria. The mean age of the study group (n = 15, female - 7) was 21.53 (± 1.0) years, and that of the control group (*n* = 18, male - 7) was 19.56 (± 0.51) years.

Study design

The current study was a prospective 2-armed study designed to observe the effect of a 10-day breathwork intervention of 40 CCB on EEG and HRV signals within and between groups. The control group did normal breathing and activities as usual, while the study group practised 40 CCB.

The study hypothesised a change in EEG and HRV readings during and after the intervention compared to baseline measures. Participants were familiarised with the measurement equipment and procedures before recording data at room temperature. Upon arrival at the laboratory, each participant relaxed for 5–10 min, after which the participant’s blood pressure, temperature, oxygen saturation and pulse rate were measured in a sitting position with eyes closed. The participant then put on the HRV chest strap (either H7 or H10) and Enobio-8 skull cap with electrodes [Figure 1] mounted at PF1-PF2, T7-T8, C3-C4 and O1-O2 regions. The HRV chest strap sensor was connected to a compatible Bluetooth-enabled software (Elite HRV) to record HRV signals transmitted on an Android phone. The Enobio-8 EEG device was connected to a WiFi signal recording application (NIC2) on the laptop. Post-readings were recorded for 10 min with 5-time events [Table 1].

Intervention

Each round of 40 CCB comprised forceful diaphragmatic breathing (four moderately deep breaths followed by one long breath) in a continuous manner without breath retention. This was then repeated for three rounds with 1 min of relaxation in between. The training was given to the study group and monitored online for 10 days. The control group was not exposed to 40 CCB breathwork and did activities as usual. During data recording, the control group did normal breathing.

Data acquisition

All data were recorded in a sitting position with eyes closed. The baseline characteristics and parameters measured are mentioned in Table 2.

EEG signals were acquired using Enobio-8 (Neuroelectrics)[21] 10–20 international system with reference linked to ear lobe electrode. The sampling rate was 500 samples/second, impedance >1G, and the noise filter was set at 50 Hz. HRV was acquired with Polar H7 and Polar H10 pro chest strap HR sensors. Each subject used only one sensor, either Polar H7 or H1, that was attached to Polar Pro straps and kept ready for randomised use. Both H7 and H10 sensors are found to have similar performance and are in best agreement with ECG (electrocardiogram).[22-24] The HRV acquired directly as RR-interval data at a sampling rate

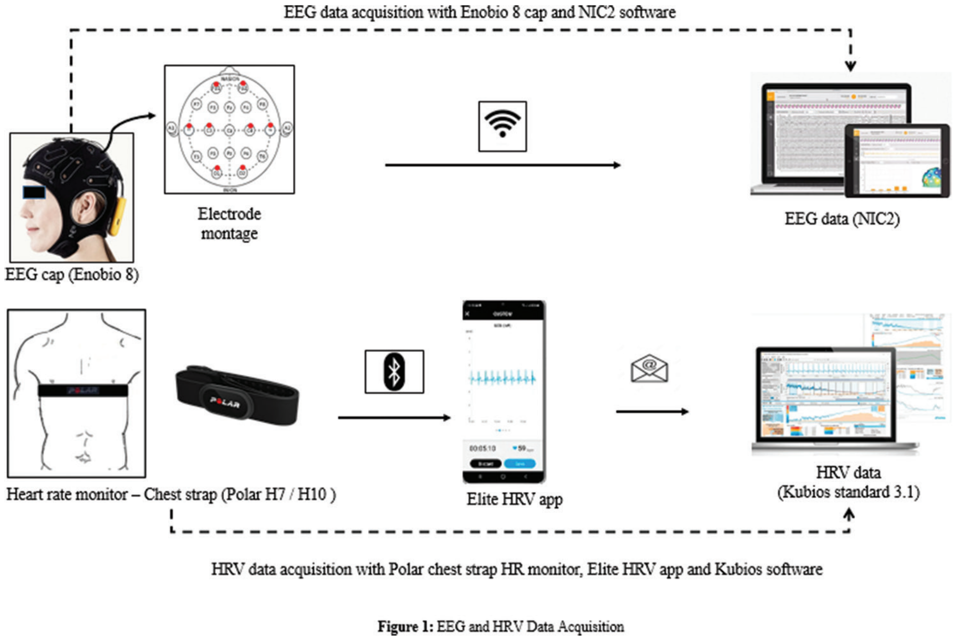

Figure 1: EEG and HRV data acquisition. EEG: Electroencephalogram, HRV: Heart rate variability.

Table 1: Study design.

Data recording (EEG+HRV) after 10-day 40 CCB practice for study group and usual activities for controls								
Pre	**During 1**	**Rest**	**During 2**	**Rest**	**During 3**	**Rest**	**Rest**	**Rest**
1st min	2nd min	3rd min	4th min	5th min	6th min	7th min	8, 9th min	10th min

Data recording process
1. EEG recording was done for 1-min durations at different time events of the study
2. *HRV was recorded continuously for 10 min and later HRV data of 1-min durations corresponding to the EEG recording time intervals as mentioned above was processed and analysed.

EEG: Electroencephalogram, HRV: Heart rate variability, *continuous recording

Table 2: Participant baseline parameters considered for the study.

Metrics	Study group (*n*=15) Mean±(SD)	Control group (*n*=18) Mean±(SD)
Age	21.53(±1.0)	19.56(±0.51)
Systole	120.6(±11.01)	116.1(±12.9)
Diastole	78.73(±6.4)	78.17(±8.6)
Oxygen saturation	97.9(±0.4)	97.5(±0.8)
Pulse rate	80.2(±10.3)	85.6(±10.5)
Body temperature	30.9(±0.9)	31.3(±0.9)

SD: Standard deviation

of 1000 Hz by the polar HR monitors was transmitted through Bluetooth to the Elite HRV app[25] on an Android phone and later downloaded in '.txt' format for further processing. Baseline1 measures were recorded for 2 min (1 min EEG and 2 min HRV). A multiparameter monitor was used to record body temperature, blood pressure, pulse rate and oxygen saturation. EEG signals were recorded through WiFi with NIC2 software. Post-readings were taken in the same process for 10 min with 5 time events [Table 1]; hence, the 6 time event readings for EEG comprised 2 baseline, 1 during and 3 after readings. HRV was recorded continuously for the entire duration (10 min) and processed for 1-min durations corresponding to the time events for which EEG was recording time intervals. The HRV recording was done continuously for administrative ease, and later, the data were processed for the corresponding EEG time interval and duration for the study.

Data sorting

Data were arranged and sorted for each channel, region and brain wave of all participants. The 6 time events were defined as T1 - Baseline1, T2 - Baseline2, T3 - During intervention, T4 - Immediately after 2nd round of breathworks, T5 - immediately after 3rd round of breathworks and T6 - after 3 min of completing 3rd round of breathworks. The average of 2 baseline readings (T1 and T2) was considered as 'Before', readings of T3 were considered as 'During', and the average of 3 readings (T4, T5, T6) were considered as 'After' readings for study (40 CCB) and control (normal breathing) groups.

EEG signal processing

EEG signals were acquired from the Enobio-8 device[21] through NIC2 software with an inbuilt algorithm for noise cancellation up to 50 Hz power interference. Signals were pre-processed with a second-order butter worth bandpass filter as an inbuilt algorithm. The power spectral density method available in the MATLAB software application was used to extract frequency domain EEG power of 5 band frequencies from raw EEG signals. Brain frequencies were classified as delta (0.5–3.5 Hz), theta (4–7 Hz), alpha (8–12 Hz), beta (13–30 Hz) and gamma (>30 Hz) and arranged for further analysis.

HRV data processing: Real-time R-R interval data were transmitted from Polar H7/H10 sensors directly to the Android phone through Bluetooth using the compatible wellness app Elite HRV, which stored R–R interval data in '.txt' format that was later exported through email and analysed with a free HRV analysis software Kubios standard (version 3.1). The recording was done continuously for the entire study duration of 10 min and later analysed in 1-min time windows corresponding to the EEG time intervals. All data were then transferred to Microsoft Excel and SPSS for statistical analysis. 10 HRV sub-parameters, including domains of time (RR, HR, RMSSD and SDNN), frequency (H.F nu, L.F nu and Total Power) and non-linear (SD1, SD2 and SD2/SD1) HRV parameters were analysed. SD1 is the standard deviation of immediate beat-to-beat interval variability, and SD2 is the standard deviation of long-term beat-to-beat interval variability.

Statistical analysis

The Statistical Package for the Social Sciences programme version 27 was used for analysis. The sorted EEG and HRV data ('Before', 'During' and 'After') were tested for normality using Kolmogorov–Smirnov's test separately for control and study groups. Corrections were made for significant differences with the inverse distribution function. *T-tests* were used for within-group and between-group statistical analyses.

RESULTS

The EEG band powers of delta, theta, alpha and beta waves decreased [Figures 2 and 3] after the 40 CCB in the study group compared to baseline; bilateral changes were not significant. Within-group [Table 3] and between-group [Table 4] changes were significant. The magnitude of EEG band power decrease [Figure 4] was higher within the study group compared to controls. There was a decrease of lesser magnitude in delta, theta and alpha band power and a significant increase in beta band power after the intervention (normal breathing) within the control group compared to the study. There was a significant decrease in alpha band power in the central region ($P < 0.05$) within the control group.

A single participant sample of MATLAB-processed EEG signals is presented in the Annexure Figure 1.

There was no significant change in the after-before reading of HRV parameters in both groups. However, in the study group, RR interval, RMSSD, SDNN, HF normalised units (n.u), Total Power and SD2 had increased. The HR, LFn.u, had decreased after the intervention compared to during

Table 3: Paired differences in EEG before and after intervention.

Brain wave	Brain region	Brain hemisphere (L=Left, R=Right)	Mean difference After-Before (Study)±SD	t-Statistic (Study) After-Before	*P*-value (Study, 2-tailed) (After-Before)	Mean difference After-Before (Control)±SD	t–Statistic (Control) After-Before	*P*-value (Control, 2-tailed) (After-Before)
Delta	O	L	−5.47±7.61	−2.8	0.01*	−1.5±10.77	−0.6	0.6
	C	R	−4.28±6.3	−2.3	0.02*	−1.5±9.1	−0.71	0.5
Theta	T	L	−3.25±3.01	−4.2	0.00*	−1.03±2.4	−1.84	0.1
	O		−3.76±4.0	−3.66	0.00*	−0.6±4.7	−0.57	0.6
	PF	R	−1.54±1.6	−3.8	0.00*	−0.9±3.5	−1.13	0.3
	O		−2.5±4.2	−2.28	0.04*	0.6±4.3	0.6	0.6
Alpha	PF	L	−1.74±2.5	−2.66	0.02*	−0.4±2.5	−0.65	0.5
	T		−2.70±2.8	−3.76	0.00*	−0.8±2.7	−1.3	0.2
	C		−0.75±3.3	−0.9	0.4	−1.2±2.1	−2.4	0.03*
	O		−2.63±2.2	−4.55	0.00*	−0.7±3.3	−0.9	0.4
	PF	R	−1.76±1.8	−3.71	0.00*	−0.9±2.3	−1.7	0.11
	T		−2.23±2.7	−3.16	0.01*	−0.4±2.1	−0.7	0.5
	O		−2.05±2.1	−3.85	0.00*	−0.9±3.1	−1.3	0.2
Beta	PF	L	−0.96±5.1	−0.73	0.5	4.3±6.5	2.81	0.01*
	T		−0.42±5.4	−0.3	0.8	5.0±6.7	3.18	0.01*
	C		−0.84±9.4	−0.35	0.8	6.8±5.2	5.54	<0.001**
	O		−5.47±7.6	−2.78	0.01*	−0.5±10.4	−0.19	0.85
	PF	R	−1.9±8.0	−0.93	0.4	3.7±4.9	3.16	0.01*
	C		−4.28±6.3	−2.63	0.02*	3.5±9.1	1.6	0.1

*$P<0.05$, **$P<0.001$, O: Occipital, C: Central, T: Temporal, PF: Pre-frontal. Most regions of delta, theta and alpha waves; left occipital and right central beta waves in study group showed significant changes. The central alpha had reduced more significantly than the study and most regions of beta wave band had increased in contrast to the study group. EEG: Electroencephalogram, SD: Standard deviation

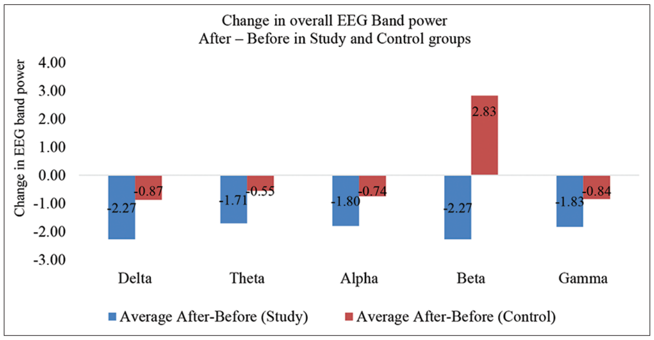

Figure 2: Overall change in EEG Band power. The delta, theta, alpha, beta and gamma band power had reduced more in study group. In contrast, beta band power increased in the control group. EEG: Electroencephalogram.

[Figure 5]. The magnitude of change was not much in the after-during reading of the control group.

DISCUSSION

The LF and HF band powers of EEG significantly decreased with a higher magnitude of decrease in the study group after the intervention compared to controls. Whereas delta and beta band power decreased in occipital and central regions,

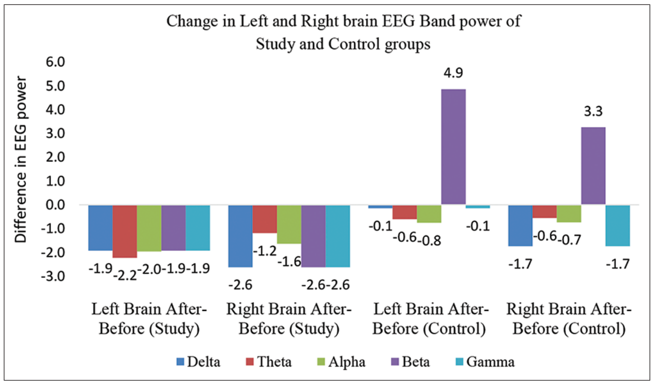

Figure 3: Overall change in EEG Band power (Left vs. Right brain). Overall, the band power reduced in both groups, but the magnitude of differences was higher and more synchronous in study group. EEG: Electroencephalogram.

theta and alpha power decreased in temporal, occipital and pre-frontal regions. The results partially corroborate to a study[26] where 45-min CCB technique in healthy adult participants decreased delta, theta and beta power bands and increased gamma power band in only experienced practitioners. In the present study, there was no significant change in gamma power, but alpha band power decreased significantly in the pre-frontal, temporal and occipital

Table 4: Between groups differences in EEG before and after intervention.

Brain Wave	Brain Region	Hemisphere (L=Left, R=Right)	Mean difference After-Before (Study)±SD	Mean difference After-Before (Control)±SD	t-Statistic (Study vs. Control) After-Before	*P*-value 2 tailed (After-Before)
Theta	T	L	−3.25±3.01	−1.03±2.4	−2.3	0.03*
Alpha	T	R	−2.23±2.7	−0.4±2.1	−2.2	0.04*
Beta	PF	L	−0.96±5.1	4.3±6.5	−2.6	0.02*
	T		−0.42±5.4	5.0±6.7	−2.4	0.02*
	C		−0.84±9.4	6.8±5.2	−2.8	0.01*
	PF	R	−1.9±8.0	3.7±4.9	−2.4	0.03*
	C		−4.28±6.3	3.5±9.1	−2.9	0.007*

*$P<0.05$, O: Occipital, C: Central, T: Temporal, PF: Pre-frontal. Significant changes were found after intervention in the temporal region for theta, alpha and beta wave bands. The magnitude of change was high in beta band. EEG: Electroencephalogram, SD: Standard deviation

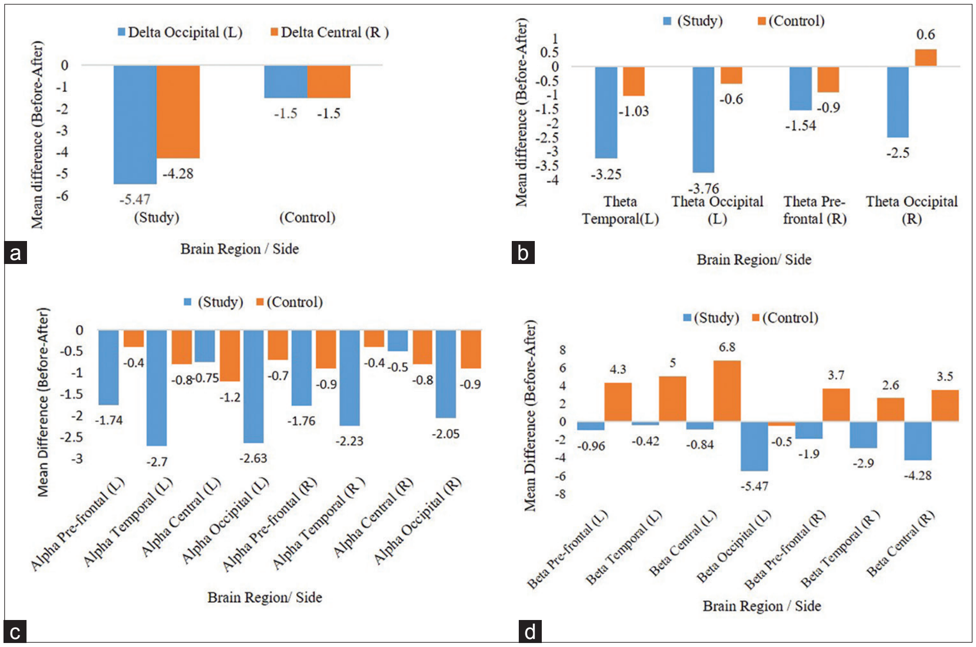

Figure 4: Illustration of significant changes in EEG band power after intervention. (a) delta, (b) theta and (c) alpha wave band power in few brain regions are reduced in both study and control groups, with higher magnitude in study group (with 40 CCB breathworks). In contrast, the (d) beta wave band power is increased in control group (with normal breathing). EEG: Electroencephalogram.

regions in the study group after the intervention compared to the baseline measure.

In the control group, alpha decreased only in the central region, and beta power increased significantly in pre-frontal, temporal and central regions. This may corroborate with findings[27] where alpha power increased in situations of anxiety, more during uncertain moments/environments as perceived by anxious individuals. Dunn *et al.*[28] observed decreased alpha power in the central region during breath concentration compared to mindfulness. Studies Huang *et al.* and Lim *et al.*[29,30] indicate varying results for alpha changes during wakeful states, though generally associated with relaxation; fatigue generates higher alpha and beta activity in adults, and beta waves in the central region are reduced during heightened concentration and immersive activities.

Hanouneh *et al.*[31] observed decreased delta and theta power in the frontal region during learning and memory retrieval, increased alpha and beta power in the frontal and decreased alpha in the parietal and temporal regions. The increase in alpha is associated with more effort during memory tasks, and a decrease in alpha power suggests a reactivation of sensory processes.[31] This can be perhaps corroborated with the current study that required participants to remember the pattern of breathing. Begić *et al.* [32] observed increased delta EEG band power (pre-frontal, frontal, parietal and occipital), theta (frontal) and beta (pre-frontal, frontal) frequencies in

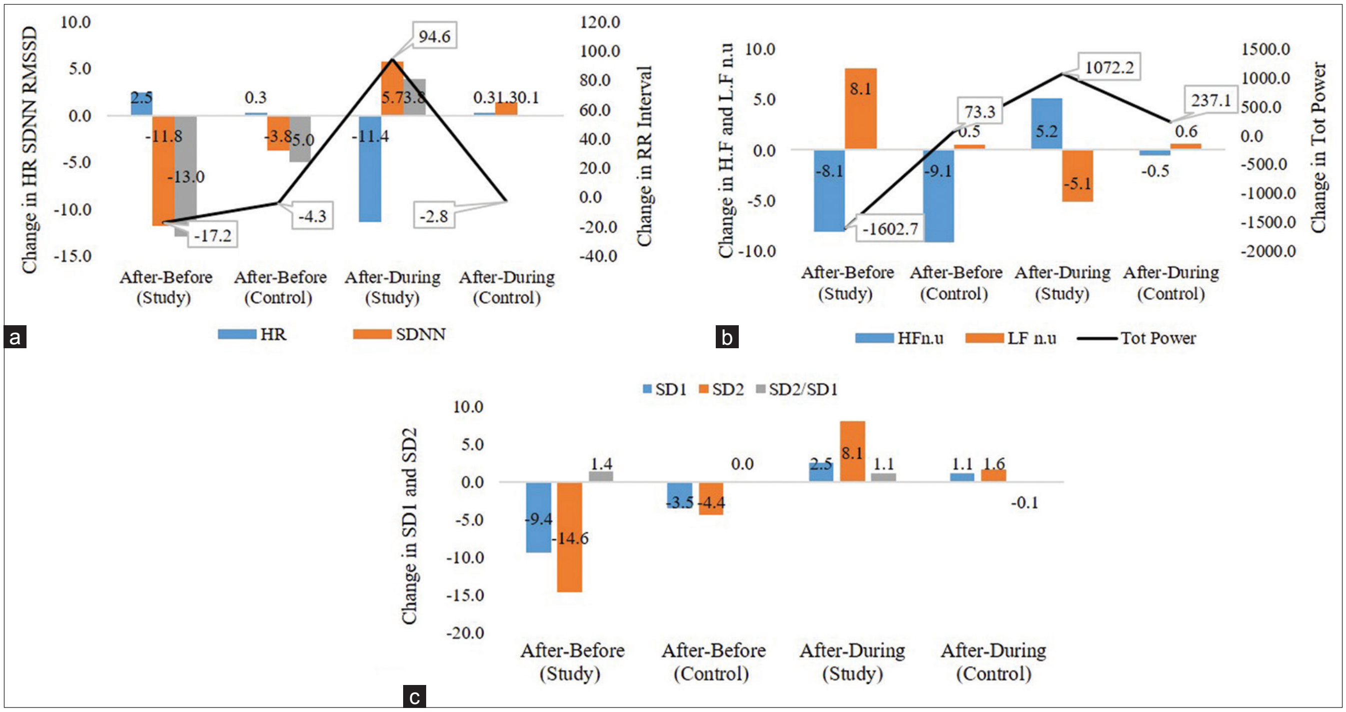

Figure 5: Change in heart rate variability parameters, HR: Heart rate, RMSSD: Root mean square of RR interval differences, SDNN: Standard deviation between normal–normal intervals, HFn.u: High-frequency normalised units, LFn.u: Low-frequency normalised units, SD1: Deviations in short-term variability of RR intervals, SD2: standard deviations in short- and long-term variability in RR intervals. (a) The positive difference in HR after-before intervention in both groups indicates an increased HR but the negative difference in after-during intervention (40 CCB breathworks) in study group indicates recovery as HR decreases immediately after breathworks. The recovery is reiterated by the positive after-during difference of SDNN, RMSSD and RR interval parameters. (b) The positive after-during difference in total power and negative change in L.Fn.u of study group indicates relaxation immediately after intervention. (c) The positive differences in SD1 and SD2 give an indication of inducing relaxation but also increased active state. HRV: Heart Rate Variability, RR intervals: distance between consecutive R-wave.

depressive compared to healthy persons. In the present study, delta, theta, and beta band powers were significantly reduced after breathwork intervention, allowing for speculation that 40 CCB can be useful in treating depression. During another technique, SKY,[33] alpha power decreased in the parieto-occipital region, and theta power increased in the centrofrontal region as rhythmic breathing progressed, delta unaffected; EEG waves slowed from alpha to theta and delta as meditation progressed;[32] during cyclic breathing of SKY, beta and alpha power increased in the frontal, midline and occipital regions. In the present study, alpha power was significantly reduced in frontal and temporal regions after 40 CCB; however, the HRV parameters showed an increase in after-during readings within the study group. Whether this can be speculated for enhancement in brain activity after breathwork practice requires further research.

Malhotra *et al.*[34] found delta increased during practice while beta and gamma activity increased after 2 months (5 min/day) of kapalabhati practice with unchanged theta and alpha EEG activity and suggested that kapalabhati has an energising, cleansing and heating effect suggesting parasympathetic withdrawal and sympathetic activation. In the present study, all EEG band powers decreased, though the increase in HRV during 3 min of recovery after 3 rounds of 40 CCB can be speculated to sway towards parasympathetic modulation. The HR, LFn.u and SD2/SD1 increased during and after the intervention, while RR, RMSSD, SDNN, H.Fn.u, total power, SD1 and SD2 decreased during the intervention in both groups compared to their respective baselines. The magnitude of change was higher in the study group compared to the control group and not significant. However, HR and LFn.u decreased in the study group compared to during the intervention, and RR, RMSSD, SDNN, H.Fn.u, SD2 and total power increased. The results corroborate with another HF breathwork study[35] where HR, systolic and diastolic blood pressure increased immediately after a 5-min kapalabhati (HF) and decreased after 20-min recovery. Similarly, HRV parameters of RR, RMSSD and H.Fn.u decreased immediately after the intervention and significantly increased after recovery. Further studies are needed to examine if effects of 40 CCB can be compared to that of kapalabhati. However, the current study allows for speculation of similar outcomes with the autonomic system on the path of recovery, balancing sympathetic modulation and parasympathetic withdrawal that were induced during 40CCB in the study group. In the control group, changes in the frequency domain of HRV (HFn.u and LFn.u) may suggest parasympathetic withdrawal and observation of simultaneous EEG beta band power increase post-intervention requires further investigation.

CONCLUSION

The current study illustrates that 10-day practice (5 min/day) of 40 CCB breathwork reduces EEG band power of delta, theta, alpha and beta waves. There is scope for further studies to determine consistency. The practice decreases HRV parameters during the breathwork and regains with recovery after 3 min, thus allowing for speculation of autonomic modulation. Further studies may help to examine the influence of 40 CCB breathworks on haemodynamic and subjective markers comparing normal healthy participants with people having mild-to-moderate conditions such as anxiety and depression.

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Ethical approval

The research/study was approved by the Institutional Review Board at Kempegowda Institute of Medical Sciences Institutional Ethics Committee, number KIMS/IEC/A071/M/2023, dated March 11, 2023.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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