Original Article

Immediate Effects of Yoga Breathing with Intermittent Breath Retention on the Autonomic and Cardiovascular Variables Amongst Healthy Volunteers

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Abstract

Background: Though breath retention is an important part of Yoga, not much is known about the physiological changes occurring following yogic breath retention. We examined the effects of 20 minutes regulated yogic breathing with intermittent breath retention (experimental session) at a frequency of 3 breaths per minute on the cardiovascular and autonomic functions.

Methods: Thirty-nine volunteers (22 females) with age-range 18 to 30 years (group mean±SD, 20.6±1.82 years) were recruited. Heart rate variability and cardiovascular variables were assessed through non-invasive blood pressure monitoring system before and after the experimental session or breath awareness (control session). The subjects were randomly assigned to either experimental or control session.

Results: There were significant reductions observed in the heart rate, stroke volume and cardiac output following the intervention. The Baroreflex Sensitivity (BRS) increased significantly following the experimental session, whereas no changes were observed following the control session. The time domain components of HRV indicated an enhanced heart rate variability following experimental session. Similar trends were observed following the control session. An increase in low frequency and decrease in high frequency components of HRV were observed following the experimental session. There was no significant change in frequency domain components following the control session.

Conclusion: The current study indicates differential autonomic modulation with enhanced BRS amongst healthy practitioners of yoga. Such yoga breathing may be useful for prevention of various metabolic disorders. The time domain components of heart rate variability suggest improvement following yoga breathing with intermittent breath retention.

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Introduction

Human respiration forms the bridge between autonomic and voluntary nervous systems since it is the only physiological system controlled by both the divisions. The impact of modulation of breathing on the autonomic and cardiovascular functions is well documented (1–4).

Breath regulation or *Pranayama* is one of the eight limbs of (*Ashtanga Yoga)* of *Patanjali* (5). Various techniques of *Pranayama* are described in *Hatha Yoga* texts (6). The texts also describe the profound effects of yoga breathing on the mind-body complex. The yoga breathing techniques include modulation of the pace of breathing, manipulation of nostrils, chanting of humming sounds, retention of breath etc. There has been growing interest in the inquiry of physiological effects of yoga and especially yogic breathing techniques in recent years (7). Several studies indicate the differential effects of various Yoga breathing techniques on autonomic functions.

In general, the practice of yoga has been found to bring balance in the autonomic functions with a trend towards parasympathetic dominance (7, 8). Various yoga breathing techniques are known to modify the cardiovascular functions (9), Baroreflex Sensitivity (10) and autonomic responses (11, 12).

Although the traditional texts of yoga emphasize on the practice of intermittent breath retention (5, 6, 13), such practice has sought very limited scientific attention. The proposed multiple health benefits of intermittent yogic breath retention include an increase in hemoglobin by increasing erythropoietin, increase in vascular endothelial growth factor leading to the formation of collaterals, reduction in blood pressure and resistance to cellular damage and thereby delayed ageing (14). A study demonstrated reduced pulse rate and increased galvanic skin resistance, following alternate nostril breathing (ANB) with intermittent breath retention (15). Another study demonstrated a significant increase in oxygen consumption while performing Ujjayi Pranayama with

breath retention for a short duration. In contrast, lowered oxygen consumption was observed with prolonged breath retention (16). Since the practice of ANB and Ujjayi Pranayama are found to influence the autonomic functions even without the practice of breath retention (10, 17, 18), the effects of intermittent breath retention remain unclear.

The physiological effects of breath retention among underwater divers have been explored. The most common physiological response of the body to voluntary breath retention is to utilize the oxygen available optimally. Such response include bradycardia, reduction in stroke volume, cardiac output, and peripheral vasoconstriction. The initial phase of breath retention alters the physiology maximally, whereas the hemodynamic changes stabilize in the later part of extended breath holding (19–21). Breath retention also leads to cerebral vasodilation and increased sympathetic tone in response to hypoxia and hypercapnia (22, 23). Further, it is demonstrated that the physiological impact of breath retention depends on the psychological status of an individual (24).

Although breath retention is practiced by both underwater divers and yoga practitioners, there are fundamental differences in the way it is practiced. Amongst the underwater divers, breath retention is performed for a maximal duration following the completion of inhalation. Yoga prescribes retention in three ways – following inhalation (*antarkumbhaka*), following exhalation (*bahyakumbhaka*) and naturally occurring breath-retention (*kevalakumbhaka*). It is also prescribed to be practiced for various durations depending on the nature of the practice of *pranayama* (13, 25).

Considering the importance of breath retention in the traditional yoga texts and lack of scientific understanding of its effects, the current study was undertaken to evaluate the effect of slow yogic breathing with intermittent breath retentions on autonomic activity, cardiovascular functions including baroreflex sensitivity (BRS) through modulation of the cardiorespiratory pathways.

Methods

Participants

Thirty-Nine volunteers (17 males + 22 females) with their ages ranging from 18 to 30 years (group mean±SD, 20.6±1.82 years) were recruited for the study. They were selected from a population of 160 students, studying various long-term courses in a Yoga University situated in South India. They had experience of practicing yoga ranging from 1 to 4 years (group mean±SD, 2.92±1.75 years). Experienced yoga practitioners were chosen for the study since breath retention is an advanced yoga practice and is not recommended to be practiced by people naïve to yoga practice. Their training in yoga included understanding yoga philosophy and practice of yoga postures (*asanas*), voluntarily regulated breathing (*pranayama*) and meditation techniques. All the participants included in the study were trained in the breathing practice assessed in the present study for 20 min/day, 6 days a week, for 8 weeks prior to the assessment. This 8 weeks of supervised training was conducted to ensure uniformity of breathing practices amongst all the participants.

Sample size

The sample size was calculated based on the effect size obtained from a previous study (26) which assessed changes in blood pressure following the practice of pranayama. It was calculated using G*Power software, Version 3.1.9.2 (27), where the Power was 0.95, $\alpha = 0.05$, the effect size (Cohen's *d*) was 1.018 and the recommended sample size resulted in being 31 participants in each group. Allowing a 20-30% attrition rate, we concluded to include 40 participants to the study.

The physical health of the subjects was assessed through routine clinical examination by a trained physician who otherwise had no role in the trial. The subjects with a history of any major illness in past 6 months especially any cardiac or respiratory disorders, consumption of any medications, tobacco, alcohol or substance abuse in any form were excluded from the study. The demographic data of the participants are presented in Table I.

TABLE I : Demographic data of the volunteers.

	Male	Female	Total
Sample size (n)	17	22	39
Age (years)	20.88±1.93	20.39±1.75	20.6±1.82
Height (cm)	168.65±6.92	158.56±6.25	162.85±8.19
Weight (Kg)	59.06±6.74	51.30±6.53	54.6±7.60
BMI (Kg/m^2)	20.73±1.74	20.36±1.86	20.52±1.80
Years of Yoga experience	3.82±2.67	4.00±2.86	3.92±2.75

Ethical consideration

The study was approved by the institutional ethics committee of the Swami Vivekananda Yoga Anusandhana Samsthana. A signed informed consent was obtained from all the participants.

Design

Following the 8-week training, the subjects were randomly assigned for the practice of yoga breathing with intermittent breath holding (experimental session) and breath awareness (control session). Half of the subjects had the experimental session on day 1 and control session on day 2 and for the rest, the order was reversed. The random allotment of the sessions was done using a web-based computer program (www.randomizer.org). Both experimental and control sessions lasted for 20 min each, which was preceded and followed by 5 min of assessment periods. The assessments were performed before and immediately following the experimental and control sessions. The time of the day was kept constant for each subject on both days. Female participants were assessed during the luteal phase of menstrual cycle (10 to 16 days after the onset of menstruation) to minimize the effect of menstrual cycle on autonomic functions (28).

Assessments

Electrocardiogram (ECG) and respiration were recorded using 16-channel human physiology system (PowerLab 16/35, ADInstruments, Australia) and blood pressure (BP) were monitored using Finapres Continuous Non-Invasive Blood Pressure (NIBP) Systems (Finapres Medical Systems B.V., Netherlands). The ECG was acquired using limb Lead

II system i.e., the electrodes were placed on the right arm and both legs (29). A standard finger cuff was connected to the left middle finger, in between the interphalangeal joints. Brachial correction was made at regular intervals as per the standard operating procedure of the instrument. The accuracy of NIBP by Fianpress Medical Systems has been standardized through comparable experiments with Intra-arterial blood pressure measurements (30, 31). Bitscope Easy v 2.0 software (Finapres Medical Systems B.V., Netherlands) was used for the recordings of NIBP. The digitized ECG data was analyzed offline to obtain the heart rate variability (HRV) spectrum. Respiration was recorded using a volumetric pressure transducer fixed around the trunk about 8 cm below the lower costal margin while the participants sat erect.

Heart rate variability

The ECG was recorded using a standard bipolar limb lead II configuration, which was digitized using a 16 bit analog to digital converter at a sampling rate of 1 KHz and was analyzed offline to obtain the HRV spectrum. Frequency domain and time domain analysis of HRV data were carried out using Lab Chart 8 (AD instruments, Australia) program, which uses Lomb-Scargle Periodogram algorithm.

Intervention

Experimental session

The experimental session included the regulated yogic breathing for 20 minutes incorporating phases of inhalation (*puraka*), internal retention of breath (*antarkumbhaka*), exhalation (*recaka*) and external retention of breath (*bahyakumbhaka*) in a ratio of 1:1:1:1 for 6 seconds each. The classic yoga texts suggest breath retention in varying ratios. The ratio for the intervention was chosen since it is considered ideal for subjects who are naïve to the practice of breath retention. The intervention was derived from a classical training methodology of pranayama suggested in the ancient text of Yoga (13). The intervals of 6 seconds were decided based on a previous study which used the similar duration of phases of breath retention along with *Nadisuddhi*

Pranayama (15). The duration of 6 seconds was ensured through verbal cues in a pre-recorded audio track.

Control session

During the control session, the participants were seated erect, performing normal breathing with breath awareness for the same duration of 20 min in the same test environment, including the audible cues. There were no adverse events reported during either the training of participants in yoga breathing with intermittent breath retention or during the recordings.

Test conditions

The recording room in the research laboratory was sound attenuated and air-conditioned in order to avoid thermal, visual or auditory disturbance. The temperature of the recording room was maintained at 25±1°C. The relative humidity during the time of the study was on average 52%. During both practice and assessments, the participants were seated comfortably, keeping the spine erect on a soft chair with backrest.

Data extraction

The following data were extracted from the 16-channel polygraph. The heart rate in beats per minute was calculated by counting the R waves of the QRS complex in the ECG. Frequency domain and time domain analysis of HRV data were performed. The energy in the HRV series in the following specific frequency bands were studied viz., Low frequency (LF) band (0.04–0.15 Hz) and highfrequency (HF) band (0.15–0.5 Hz). According to guidelines, LF and HF band values were expressed as normalized units. The LF/HF ratio was also calculated. The following components of time domain HRV were analyzed: (i) SDNN (the standard deviation of NN intervals), (ii) the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD), (iii) the proportion derived by dividing NN50 by the total number of NN intervals (pNN50). The respiratory rate in cycles per minute (cpm) was calculated by counting the total breath cycles.

Brachial artery systolic (SBP) and diastolic pressures (DBP) were extrapolated from finger arterial pressure through the use of a height correction unit and waveform filtering and level correction methods. Mean arterial pressure (MAP), SBP and DBP were expressed in mmHg. The computed measurements of Stroke volume (SV), cardiac output (CO) from the arterial BP and HR has been found reliable when compared to Modelflow-derived CO (32). The Total Peripheral resistance (TPR) estimation from the computed CO was also found to be valid (33). Another variable of interest, Baroreflex Sensitivity (BRS) was estimated from the spontaneous HRV and BP variability (BPV) measured by the Finapress method (34).

Data analysis

The data were analyzed by the statistician using Statistical Package R version 3.2.4 (www.r-project.org). Repeated measures analyses of variance (RM-ANOVA) were performed with two Within-Subjects factors, i.e., (i) Sessions with two levels; intervention and control and (ii) States with two levels, pre and post intervention.

Results

The results are presented as the group mean and standard deviation for the autonomic and the cardiovascular variables (Table II).

Following the experimental session, an increase in SDNN, RMSSD, pNN50 of the time domain variables of HRV was observed. A significant increase was also noted in LFnu, Total Peripheral Resistance and Baroreflex Sensitivity following the experimental session. We also found a reduction in HFnu, MAP, SV and CO in the experimental session. The heart rate reduced following both experimental and control sessions. After control session, an increase in RMSSD, pNN50, SBP and SV was noted. A reduction was observed in CO following the control session. No changes were observed in the frequency domain variables of HRV and Baroreflex Sensitivity following the control session. Although there was reduction noted in the respiratory rate in both groups, the changes were non-significant. Also, Pre-Post breath rates were incidentally observed to be similar for both the sessions.

Repeated measures analysis of variance

The significant changes in the components of HRV and cardiovascular variables are presented in Table III.

Post hoc analyses with Bonferroni adjustment

There was a significant reduction in HR (P<0.001,

TABLE II : Changes in the Heart Rate Variability and Cardiovascular variables before and following the experimental and control sessions.

	Experimental session		Control session	
	Pre	Post	Pre	Post
Heart rate (beats/min)	78.54±10.54	74.92±9.25***	76.67±9.58	73.94±9.29**
SDNN (ms)	64.47±6.27	74.76±29.20**	65.67±28.02	70.84±28.57
RMSSD (ms)	45.98±22.90	52.39±24.96***	49.23±23.22	54.81±23.93*
pNN50 (% units)	21.26±16.65	24.99±16.49**	25.20±17.78	30.67±17.75**
LFnU	61.72±17.55	67.51±15.77*	57.38±18.96	55.56±20.55
HFnU	38.53±17.16	32.65±15.27*	42.48±18.57	43.71±19.65
LF:HF	2.58±2.72	3.10±2.70	2.07±1.96	2.11±2.59
Respiratory Rate (cycles/min)	15.42±3.46	14.63±4.03	16.18±3.71	15.38±4.11
Systolic BP (mmHg)	102.10±12.21	101.04±11.39	104.44±10.91	106.83±11.86***
Diastolic BP (mmHg)	59.33±8.76	58.49±.30	60.88±8.46	60.61±8.23
Mean Arterial pressure (mmHg)	77.88±10.12	76.35±9.54*	79.52±9.10	79.59±8.99
Stroke Volume (ml)	68.35±15.83	66.20±15.75*	70.36±12.04	72.22±11.41***
Cardiac output (l/min)	5.27±1.20	4.88±1.05***	5.32±0.85	5.26±0.81**
Total Peripheral Resistance	0.99±0.37	1.04±0.39***	1.07±0.70	0.99±0.36
Baroreflex Sensitivity (ms/mmHg)	14.56±.55	15.81±5.71**	16.24±8.83	16.50±7.75

Repeated Measures Analyses of Variance with post hoc Bonferoni adjustment, *=p<0.05, **=p<0.01, ***=p<0.001
SDNN: standard deviation of NN intervals; RMSSD: root of the mean of the sum of the squares of differences between adjacent NN intervals; pNN50: proportion derived by dividing NN50 by the total number of NN intervals.

TABLE III : Summery of the Repeated Measures Analysis of Variance (RM-ANOVA) showing statistically significant results.

Variables	Factor	F Value	df	Level of significance
LFnu	Sessions	13.81	1, 38	0.01
HFnu	Sessions	13.61	1, 38	0.01
LF/HF ratio	Sessions	6.17	1, 38	0.05
pNN50	Sessions	5.17	1, 38	0.05
Systolic BP	Sessions	81.20	1, 38	0.001
Diastolic BP	Sessions	213.62	1, 38	0.001
Mean arterial pressure	Sessions	18.84	1, 38	0.001
Cardiac output	Sessions	612.38	1, 38	0.001
Stroke volume	Sessions	6.81	1, 38	0.05
Total peripheral resistance	Sessions	581.17	1, 38	0.001
Heart rate	States	37.96	1, 38	0.001
RMSSD	States	15.79	1, 38	0.001
pNN50	States	16.77	1, 38	0.001
SDNN	States	13.04	1, 38	0.01
Systolic BP	States	81.33	1, 38	0.001
Diastolic BP	States	732.29	1, 38	0.001
Mean arterial pressure	States	343.79	1, 38	0.001
Cardiac output	States	13.83	1, 38	0.01
Stroke volume	States	22.67	1, 38	0.001
Total peripheral resistance	States	580.45	1, 38	0.001
HFnu	Sessions x states	4.12	1, 38	0.05
Systolic BP	Sessions x states	81.31	1, 38	0.001
Diastolic BP	Sessions x states	1030.13	1, 38	0.001
Mean arterial pressure	Sessions x states	292.73	1, 38	0.001
Cardiac output	Sessions x states	14.07	1, 38	0.01
Total peripheral resistance	Sessions x states	617.01	1, 38	0.001

post hoc analyses following ANOVA), HFnu (P<0.05), MAP (P<0.05), SV (P<0.05), CO (P<0.001), whereas increase was noted in LFnu (P<0.05), SDNN (P<0.001), RMSSD (P<0.01), pNN50 (P<0.01) TPR (P<0.001) and BRS (P<0.01) following the experimental session. Reductions in HR (P<0.01), CO (P<0.01) and TPR (P<0.001) whereas increase in RMSSD (P<0.05), pNN50 (P<0.01), SBP (P<0.001) and SV (P<0.001) were observed following the control session.

Discussion

This study investigated the effect of yoga breathing with intermittent breath holding on frequency and time domain variables of heart rate variability (HRV) and cardiovascular functions in healthy yoga practitioners. To the best of our knowledge, this is the first attempt to scientifically explore the effects of isolated yoga breathing with breath retention among healthy volunteers. The earlier studies where yogic breath retention was used, it was in combination with other yoga breathing techniques (15, 16, 35).

HRV is the physiological phenomenon of variation in the time interval between heartbeats. It is measured by the variation in the beat-to-beat intervals (36). HRV is widely utilized to interpret the cardiac autonomic regulation following various yoga practices (7). HRV is the pattern of several overlapping oscillatory frequency components. Three components of the frequency domain analyses of HRV have been identified viz., the high frequency (0.15–0.4 Hz), low frequency (0.05–0.15 Hz), and very low frequency (0.005–0.05 Hz). In general, LF component is correlated with the activity of sympathetic and parasympathetic nervous system whereas HF component with parasympathetic activity. The physiological interpretation of VLF component is unclear (36). It is also observed that, high-amplitude peaks in the LF range during rhythmical slow breathing may reflect resonance characteristics of the cardiovascular system where respiratory sinus arrhythmia interacts with the baroreflex (37). Breathing at such resonant frequency may increase HRV and be reflected in large increases in the LF band and simultaneous decreases in the HF band. The findings of the spectral analysis of HRV of the current study indicate an increase in LF and a corresponding reduction in HF, with enhanced baroreflex sensitivity. These changes, thus, may be attributed to breathing at a very slow rate of 2.5 Hz.

The findings are similar to earlier yoga studies demonstrating an increase in LF with slow yoga breathing (38). Breathing at such slow rate imitating the resonant frequency is found to influence the heart rate and blood pressure oscillations and thus enhance the overall HRV (39) and subsequent reduction in heart rate and blood pressure (40). Yet, the blood pressure changes were nonsignificant in the current study. No changes in the frequency components of HRV were observed following the control session.

Among the time-domain variables, SDNN is an indicator of overall heart rate variability, whereas RMSSD and pNN50 are associated with vagal tone (36). The changes in the time domain components of the HRV were similar following both experimental and control sessions with an increase in SDNN, RMSSD and pNN50, thus indicating an enhanced HRV. Yet, the magnitude of change was higher following the experimental session. However, the reason for the change following the experimental session remain unclear, whether it was due to intermittent breath retention or slow breathingalone. Since, the participants were long-term yoga practitioners, and performing breath awareness during the control session, they might have entered a meditative state and thus modulating the HRV. Such enhanced HRV is common among long-term yoga practitioners (7). Similar changes in time-domain variables of HRV were observed in a previous study in participants practicing breath awareness (41).

We also found a significant increase in the Baroreflex Sensitivity following the 20 min experimental session. Our findings are consistent with earlier studies elucidating the influence of yogic breathing techniques on Baroreflex Sensitivity in healthy (10) as well as Clinical population with essential hypertension (42) and chronic heart failure (43). Such gain in the baroreflex sensitivity may also be due to slow breathing in the experimental session at the resonant frequency of about 2.5 Hz. Also, the earlier studies attribute the gain in Baroreflex Sensitivity to increased vagal tone, indicated by a gain in RMSSD and pNN50 as well as reduced heart rate. Arterial baroreceptor activity and respiratory sinus arrhythmia are interrelated (44) and therefore the increase in Baroreflex Sensitivity could be attributed to enhanced

HRV following the experimental session. Jerath et. al. propose the action of inhibitory signals and hyperpolarizing current within neural and non-neural tissue activation of slowly adapting stretch receptors, responsible for modulation of the activity of the cardiorespiratory centers (45).

Reduced heart rate variability and baroreflex sensitivity is found to be a risk factor for cardiovascular diseases (46, 47), diabetes mellitus (48, 49) and various metabolic syndromes (50). The enhanced heart rate variability and baroreflex sensitivity observed following the yoga breathing assessed in the present study may indicate its role in preventing such disorders. Future studies may incorporate clinical population to assess the effect of yoga breathing with intermittent breath retention on the cardiac autonomic regulation.

We also found an increase in the total peripheral resistance and LFnu indicative of a possible sympathetic shift in the autonomic activity. These changes may be due to the very nature of the intervention, which includes focused attention on the verbal cues and constant synchronization of the breathing with it. The nature of intervention needed constant attention, which may be responsible for the selective sympathetic arousal. An earlier study of yoga breathing with breath-retention for short duration indicated an increase in oxygen consumption, which might be considered similar to the results of the present study (16). Also, intermittent hypoxia created in the experimental session would contribute to enhanced sympathetic tone (23). Despite the sympathetic arousal, the gain in baroreflex sensitivity may be attribute to inhibition of chemoreflex mechanisms due to slow breathing (51). Also, long term yoga practitioners demonstrate a generalized reduction in chemoreceptor sensitivity (52). Slow breathing possibly leads to a generalized attenuation in the excitatory pathways for respiratory and cardiovascular systems. Both respiratory and cardiovascular systems share similar control mechanisms, thus alterations in breathing may be responsible for the cardiovascular changes (53).

The reduction in cardiac output and stroke volume may be a result of body's compensatory mechanism

due to intermittent breath retention along with very slow breath. Also breathing at resonant frequency has been shown to enhance the gaseous exchange and oxygen saturation (54), thereby reducing the overall circulatory load. The changes observed in the current study following the intervention are similar to those found following voluntary breath retention in swimmers and divers, which include bradycardia, reduction in stroke volume, cardiac output, and peripheral vasoconstriction (19). However, in the current study, the practice of breath-retention was intermittent and short term instead of the maximal breath retention as practiced by the divers.

Although we could demonstrate differential changes in the autonomic and cardiovascular activity following yoga breathing with intermittent breath retention, the examination of the exact underlying mechanisms was beyond the scope of the study. Assessments during the practice of yoga breathing with intermittent retention may bring clarity on the underlying mechanisms. Lung volume and the partial pressure of CO_2 (PaCO2) are known to influence the HRV spectrum in conscious subjects (55). We could not control the lung volume as well as assess the PaCO2 in the current study. Thus, future studies may be planned to assess the effects of lung volume and PaCO2 in yoga breathing techniques. Future studies may also include neuroimaging techniques focusing on the neural centers for the vagus nerve to understand the underlying mechanisms. We did not examine the long-term effects of the practice of yoga breathing with intermittent breath retention. Our study population was limited to healthy young volunteers,

with training in yoga. Future studies may be designed to understand the effects of yoga breathing with breath retention in different populations and clinical setting.

Conclusion

The current study indicates differential autonomic modulation with enhanced Baroreflex Sensitivity along with selective sympathetic activation amongst healthy practitioners of yoga. The time domain components of heart rate variability suggest improvement following yoga breathing with intermittent breath retention and a similar trend following normal breathing with breath awareness. Such yoga breathing may be useful for prevention of various metabolic disorders including heart diseases and diabetes mellitus. Further studies using neuroimaging techniques in different populations could be used to understand the exact mechanisms involved in the practice of yogic breath retention and its specific effects.

Compliance with ethical standards

Disclosure of potential conflicts of interest: The authors declare no conflict of interests.

Research involving Human Subjects: The study has been approved by the institutional ethics committee and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent: Written informed consent was obtained from all the participants.

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