CHAPTER-5

Materials and Methods

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5.0 MATERIALS AND METHODS

5.1 SUBJECTS:

5.1.1 Sample Size:

A total of fifty subjects (n = 50) were included in the study which includes,

Phase 1: Thirty subjects [*Yoga* practitioners (n = 15) and Non *Yoga* practitioners (n = 15)**]**

Phase 2: Twenty healthy *Yoga* practitioners (n = 20)

5.1.2 Sample Size Calculation:

The sample size calculation was made based on a previous study on "cerebrovascular hemodynamic changes during different *Pranayama* techniques. "The sample size was calculated using Gpower 3.1.9.2 software with the effect size = 0.953, alpha value = 0.05 and power (1-β) = 0.95, and the actual sample size calculated was (n = 14). Assuming the chances of getting the poor signals or challenges in getting the proper signals in TCD the sample size was kept as (n = 20).

5.1.3 Source of the Subjects:

- Subjects (*Yoga* practitioners) were recruited from Swami Vivekananda *Yoga* Anusandhana Samsthana, Prashanthi Kuteerum, Jigani, Bengaluru
- Subjects (Non *Yoga* practitioners) were recruited from Sushrutha Ayurvedic Medical College, Prashanthi Kuteerum, Jigani, Bengaluru

5.1.4 Inclusion and Exclusion Criteria:

All the subjects were recruited based on the following inclusion and exclusion criteria.

Inclusion Criteria for *Yoga* practitioners: (Phase 1 & Phase 2)

- Normal healthy volunteers with age range from 18 to 35 years
- Gender: Male and females
- Body mass index (BMI): 18.5 – 24.99 kg/m^2
- Subjects who are willing to participate in the study.
- Subjects who are experienced in practicing *Yoga* techniques including *Pranayama* for more than one year

Exclusion criteria for *Yoga* practitioners: (Phase 1 & Phase 2)

- Subject with a history of any systemic and mental illness.
- Subjects with a history of regular medication for any diseases
- Subjects with the history of chronic substance abuse, smoking or alcoholism
- Subjects who is unable to perform *Pranayama*

Inclusion Criteria for Non *Yoga* Practitioners: (Phase 1)

- Normal healthy volunteers with age range from 18 to 35 years
- Gender: Male and females
- Body mass index (BMI): 18.5 – 24.99 kg/m^2
- Subjects who are willing to participate in the study.
- Subjects who does not have experience in practicing *Yoga* techniques including *Pranayama*

Exclusion criteria Non *Yoga* Practitioners: (Phase 1)

- Subject with a history of any systemic and mental illness.
- Subjects with a history of regular medication for any diseases
- Subjects with the history of chronic substance abuse, smoking or alcoholism

5.1.5 Ethical considerations:

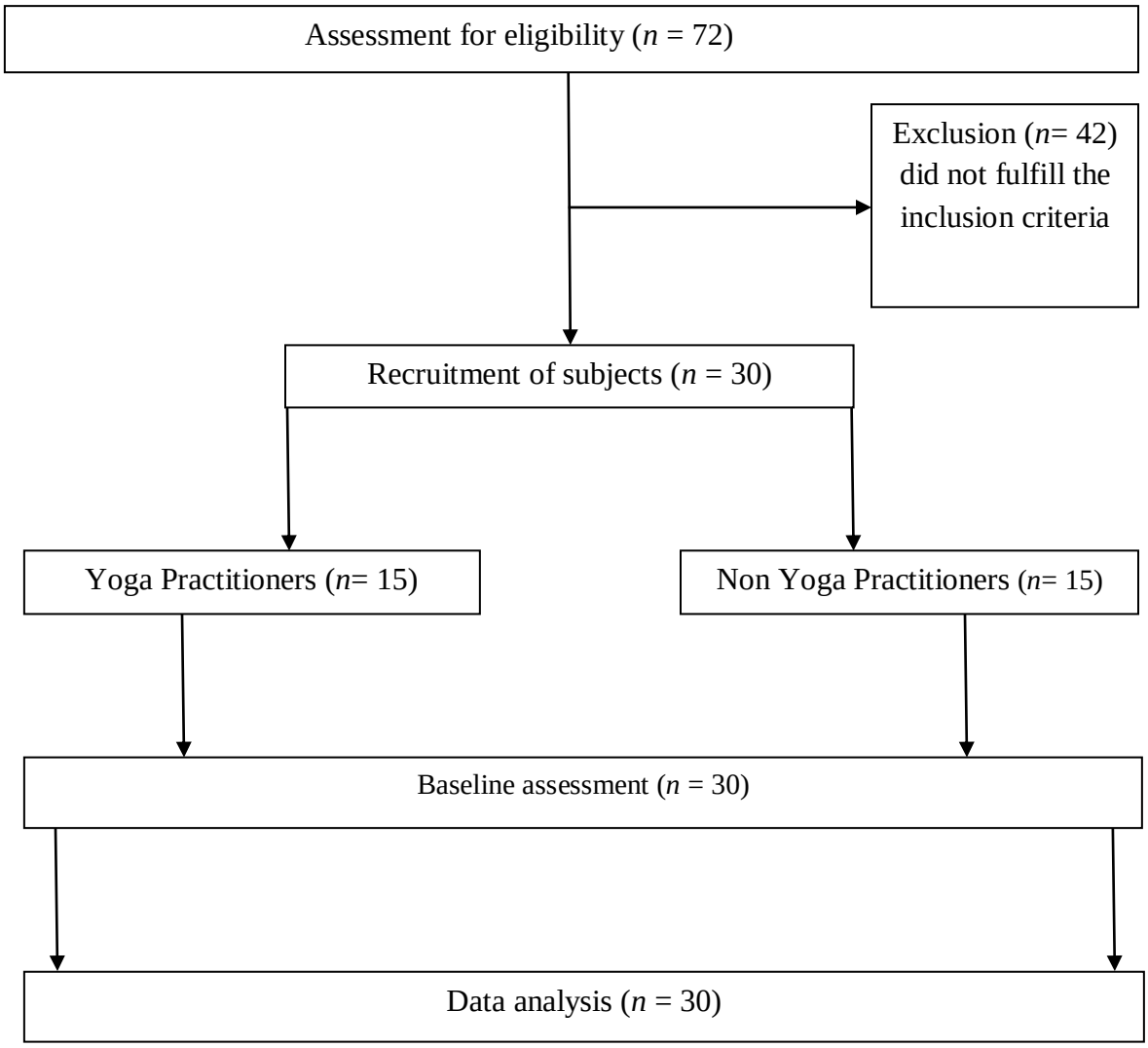
An approval has been obtained from the Institutional Ethics Committee, S-VYASA University, Bengaluru. All the participants were explained about the study protocol in detail and a signed written informed consent was obtained from each participant who was willing to participate in the study.

5.2 DESIGN OF THE STUDY:

Phase 1:

A *cross sectional design* was adopted for the phase 1 of the study. In this design, assessments of cerebrovascular hemodynamics and cardiovascular functions were assessed for both *Yoga* practitioners (*n* = 15) and non *Yoga* practitioners (*n* = 15) at one point of time without any intervention.

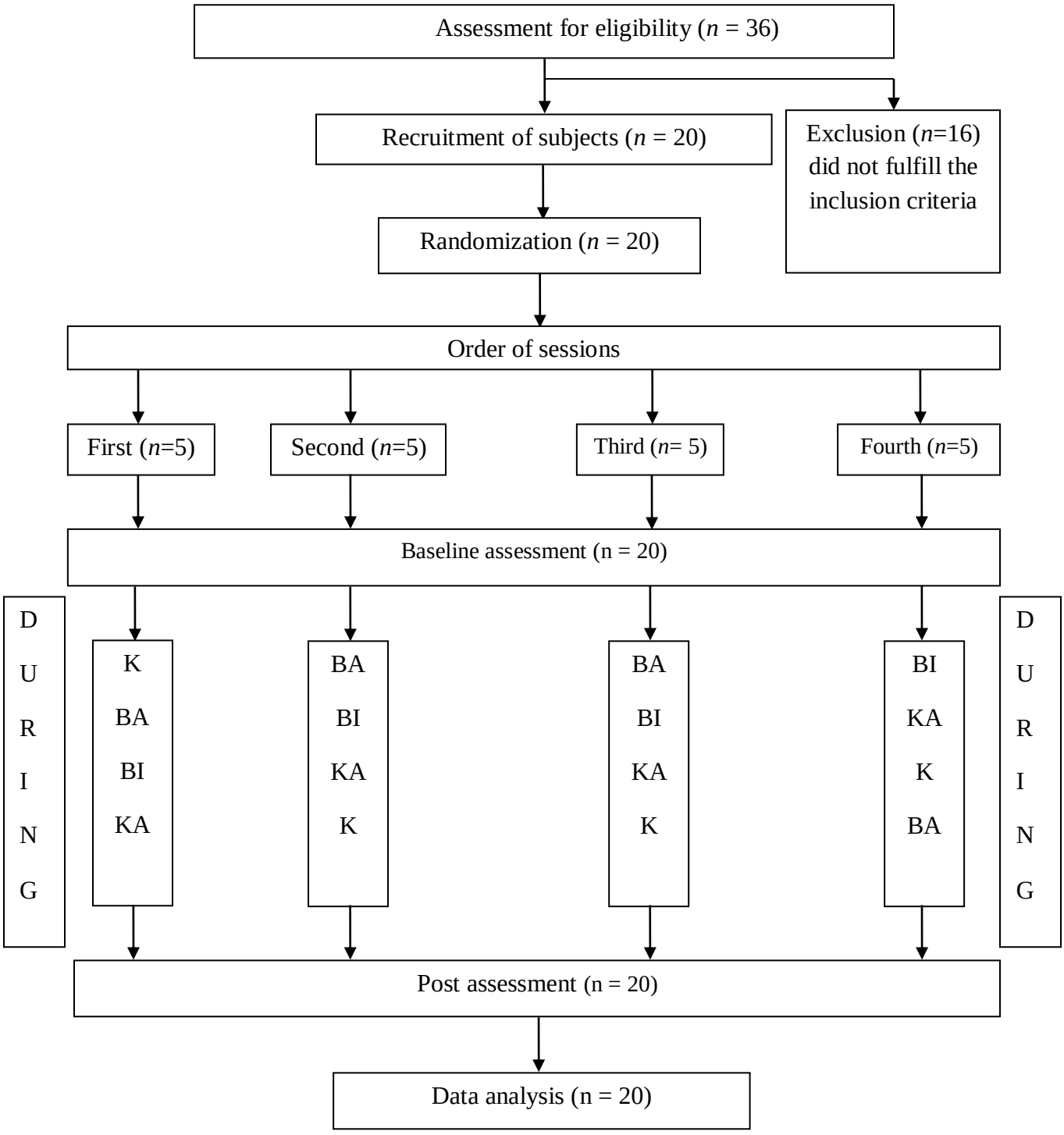
Figure 1: Trial Profile for Phase 1



Phase 2:

A *self as control design* was adopted for the phase 2 of the study. In this design, the subjects (*n* = 20) were asked to perform selected *Pranayama* techniques & a *Yogic Kriya* in 4 different orders. In first order (*n*= 5) all the subjects started with *Kumbhaka* (internal and external) followed by *Bhastrika, Bhramari* and finished with *Kapalbhati*; In second order (*n*= 5) all the subjects were started with *Bhastrika* followed by *Bhramari*, *Kapalbhati* and finished with *Kumbhaka* (internal and external);In third order (n = 5) all the subjects started with *Bhramari* followed by *Kapalbhati*, *Kumbhaka*(internal and external) and finished with *Bhastrika*; In fourth order (n=5) all the subjects started with *Kapalbhati* followed by *Kumbhaka* (internal and external), *Bhastrika* and finished with *Bhramari* with the interval of 5 minutes between each *Pranayama*. Baseline assessment was taken at rest before starting the practice, whereas during and post assessments were taken during and immediately after each *Pranayama* techniques & a *Yogic Kriya*.

Figure 2: Trial Profile for Phase 2



*K = *Kumbhaka*; BA = *Bhastrika*; BI = *Bhramari*; and KA= *Kapalbhati*

5.3 ASSESSMENTS:

Phase 1 and Phase 2:

Height: By using standard measuring tape, height in cm of each subject was measured.

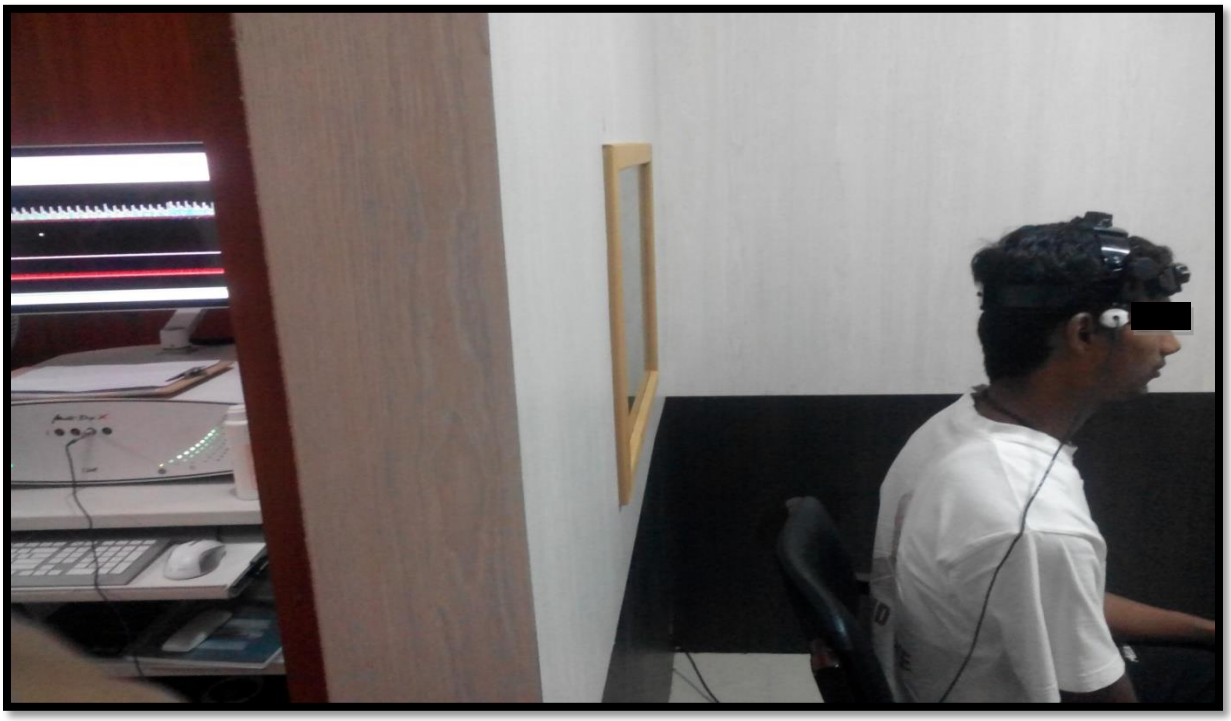
Weight: By using standard weighing machine, the weight in kg of each subject was measured.

Body mass index (BMI): It has been derived by using height and weight in the formula of weight in kg divided by height in meter square.

Phase 1:

Cerebrovascular Haemodynamic Changes:

Cerebrovascular hemodynamic changes were monitored in right middle cerebral artery (MCA) with Transcranial Doppler (TCD) (Multi-Dop X, DWL, Germany). A 2-MHz TCD ultrasound transducer probe was placed with the use of a head frame in the right temporal area just above the zygomatic arch and in front of the tragus of the ear. The transducer was adjusted to get consistent flow spectra between 45 and 60-mm. Right MCA was chosen only for consistency and technique of fixing the head frame and monitoring transducer. TCD parameters recorded during the monitoring included peak systolic velocity (PSV), end diastolic velocity (EDV) mean flow velocities (MFV) in cm/s and Pulsatility index (PI) (Bathala et al., 2013).These measurements were recorded for the duration of 60 seconds for both *Yoga* and non *Yoga* practitioners. All subjects were asked to refrain from drinking coffee/tea for three hours before the study.

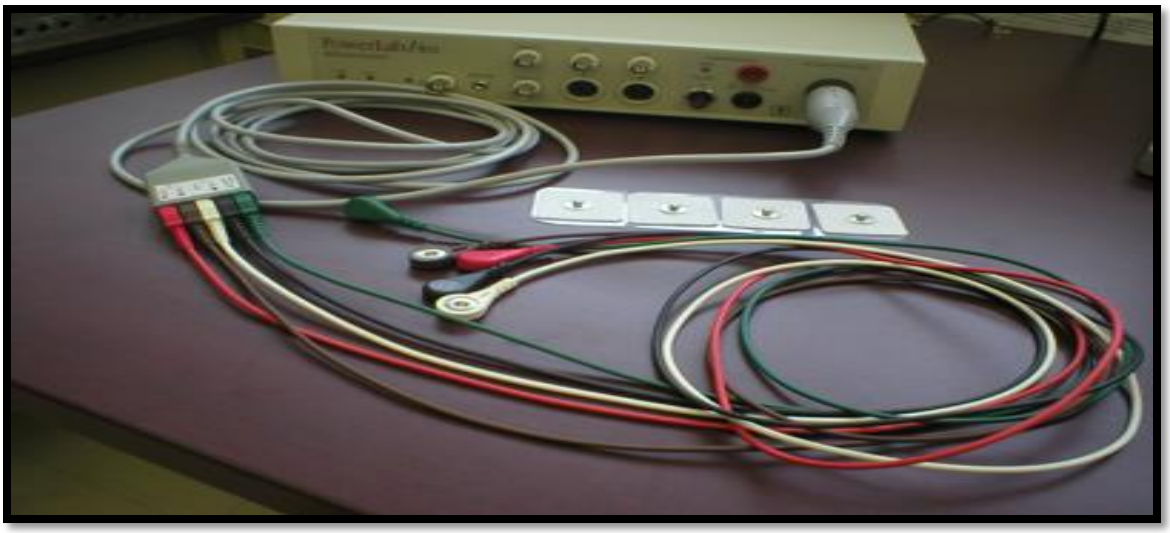


Autonomic variables:

A 4-channel polygraph (MP 100, Biopac Student Lab, BIOPAC System Inc, U.S.A.) was used to record the electrocardiogram (ECG) and respiration.

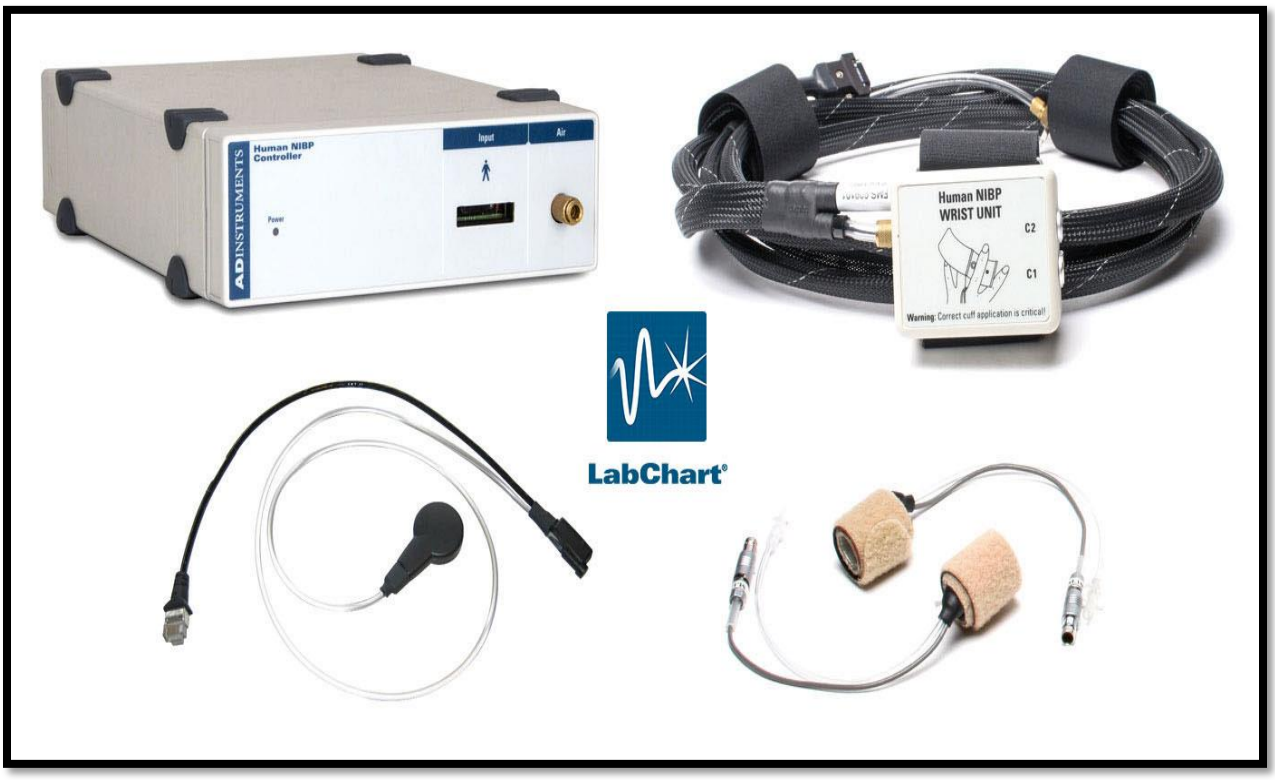
***Heart rate**:*

ECG was recorded using standard limb lead I configuration. The ECG was digitized using a 12 bit analog-to-digital converter (ADC) at a sampling rate of 1024 Hz.



Cardiovascular Functions:

A beat to beat changes of the cardiovascular variables such as systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (HR), stoke volume (SV), left ventricular ejection time (LVET), cardiac output (CO), pulse interval (PI), and total peripheral resistant (TPR) were assessed in sitting position using non-invasive blood pressure monitoring system (Finapres Continuous Non Invasive Blood Pressure Systems, Netherland). A finger cuff of suitable size was placed on the left middle finger, in between the inter-phalangeal joints. A Non-invasive blood pressure cuff was placed on the upper arm of the same hand at the level of the heart and the marker on the cuff was directly above the brachial artery. The hand was placed at the knee and flexed at the elbow. Brachial correction was also made for each subject before assessment. Assessments were taken at rest for the duration of five minutes.



Phase 2:

Cerebrovascular Haemodynamic Changes:

Cerebrovascular hemodynamic changes were monitored in right middle cerebral artery (MCA) with Transcranial Doppler (TCD) (Multi-Dop X, DWL, Germany). A 2-MHz TCD ultrasound transducer probe was placed with the use of head frame in the right temporal area just above the zygomatic arch and in front of the tragus of the ear. The transducer was adjusted to get consistent flow spectra between 45 and 60-mm. Right MCA was chosen only for consistency and technique of fixing the head frame and monitoring transducer. TCD parameters recorded during the monitoring included peak systolic velocity (PSV), end diastolic velocity (EDV) mean flow velocities (MFV) in cm/s and Pulsatility index (PI) (Bathala et al., 2013).

These measurements were recorded at baseline (0 sec) and every 15 seconds during the practice of *Bhastrika*, Internal *Kumbhaka* & *Kapalbhati* i.e. 15, 30, 45, and 60 seconds. During external *Kumbhaka* measurements were taken at baseline (0 sec), 10, 20, and 30 seconds. During *Bhramari Pranayama* measurements were taken at baseline (0 sec), and every 1 minute i.e. 1, 2, 3, 4, and 5 minutes. The post-test assessments of all the *Pranayama* techniques & *Kapalbhati* were taken immediately after each *Pranayama* at every 15 seconds for the duration of 60

seconds. All subjects were asked to refrain from drinking coffee/tea three hours before the study.

Autonomic variables:

A 4-channel polygraph (MP 100, Biopac Student Lab, BIOPAC System Inc, U.S.A.) was used to record the electrocardiogram (ECG) and respiration.

***Heart rate**:*

ECG was recorded using standard limb lead I configuration. The ECG was digitized using a 12 bit analog-to-digital converter (ADC) at a sampling rate of 1024 Hz.

Respiratory Rate:

Respiration was recorded using a volumetric pressure transducer fixed around the chest as the subject was seated erect. Care was taken to adjust the strap such that full inhalation is not restricted. This was performed to confirm absence of passive inhalation during the practice of *Kumbhaka* (both internal and external).

Cardiovascular Functions:

A beat to beat changes of the cardiovascular variables such as systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (HR), stoke volume (SV), left ventricular ejection time (LVET), cardiac output (CO), pulse interval (PI), and total peripheral resistant (TPR) were assessed in sitting position using non-invasive blood pressure monitoring system (Finapres Continuous Non Invasive Blood Pressure Systems, Netherland). A finger cuff of suitable size was placed on the left middle finger, in between the inter-phalangeal joints. A Non-invasive blood pressure cuff was placed on the upper arm of the same hand at the level of the heart and the marker on the cuff was directly above the brachial artery. The hand was placed at the knee and flexed at the elbow. Brachial correction was also made for each subject before assessment. Assessments were taken at rest before starting of the *Pranayama* (baseline), during and after each practice.

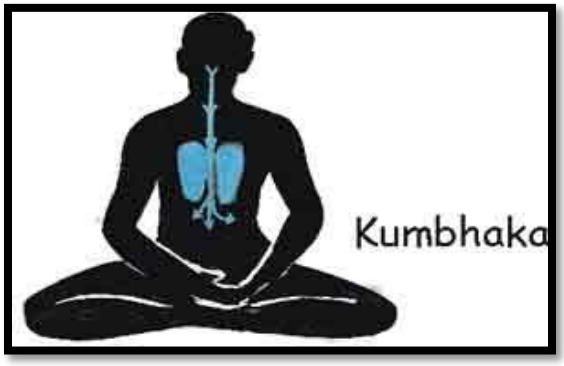
5.4 INTERVENTION:

5.4.1 *Kumbhaka*:

pävano badhyate yena manastenaiva badhyate|
manaçca badhyate yena pavanastena badhyate||

(Chapter 2, Verse 40, Hatha *Yoga* Pradipika)

- *Internal breath retention (Antar-Kumbhaka) session:* The subjects were asked to inhale slowly and deeply through both the nostrils and then to retain the breath internally for 1 minute. This was followed by normal exhalation. This was one round and the same was repeated for 3 rounds with a gap of 1 minute between each round during which subjects were allowed to relax with normal breathing (rest period). Hence, the total duration including the rest period will be of 5 minutes.
- *External breath retention (Bahir-Kumbhaka) session:* The subjects were asked to exhale fully through both the nostrils and then to retain the breath internally for 30 seconds. This was followed by normal exhalation. This was one round and the same was repeated for 3 rounds with a gap of 1 minute between each round during which subjects were allowed to relax with normal breathing (rest period). Hence, the total duration including the rest period will be of 3 minutes 30 seconds.

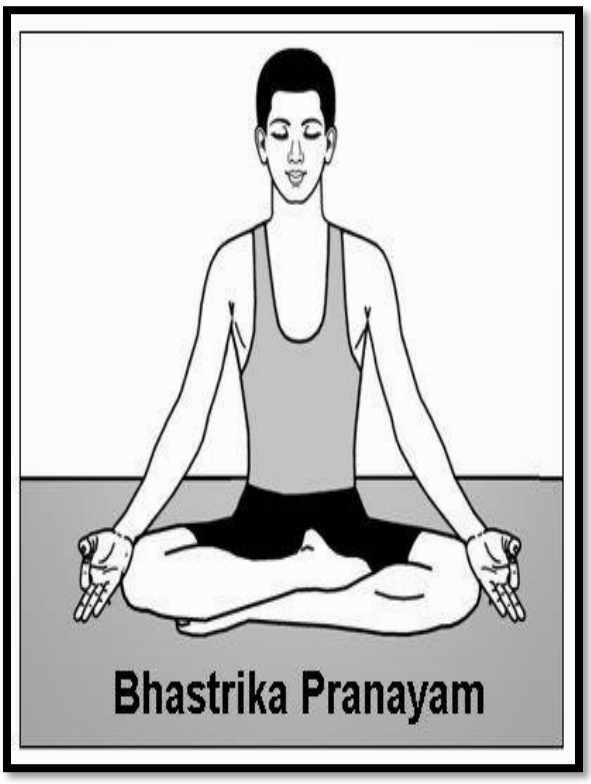


5.4.2 *Bhastrika*:

punarvirecayettadvatpürayecca punaù punaù|
yathaiva lohakäreëa bhasträ vegena cälyate||

(Chapter 2, Verses 62, Hatha *Yoga* Pradipika)

The subjects were asked to inhale and exhale forcefully through both nostrils by producing a sudden and forceful contraction of the abdomen while exhaling and relaxing it while inhaling at the rate of 1 cycle/second for the duration of 1 minute. This was one round and the same was repeated for 3 rounds with a gap of 1 minute between each round during which subjects were allowed to relax with normal breathing (rest period). Hence, the total duration including the rest period will be of 5 minutes.

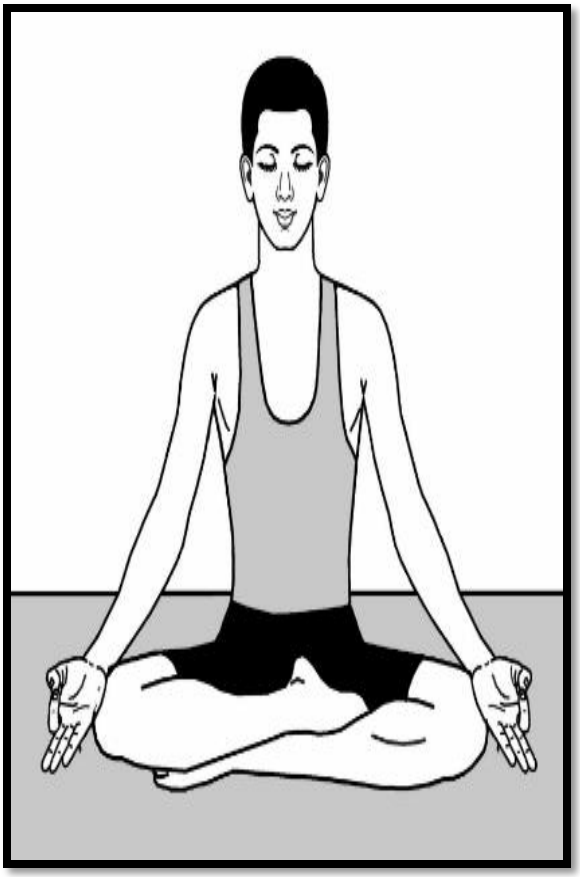


5.4.3 *Bhramari*:

vegädghoñaà pürakaà bhåìga-nädaà
bhåìgé-nädaà recakaà manda-mandam |
yogéndräëamevamabhyäsa-yogäccitte jätä käcidänanda-lélä ||

(Chapter 2, Verse 68, Hatha *Yoga* Pradipika)

Subjects were asked to perform inhalation through both nostrils and while exhaling they were asked to produce the sound of a humming bee at the rate of 3 cycles/ minute for the duration of 5 minutes.

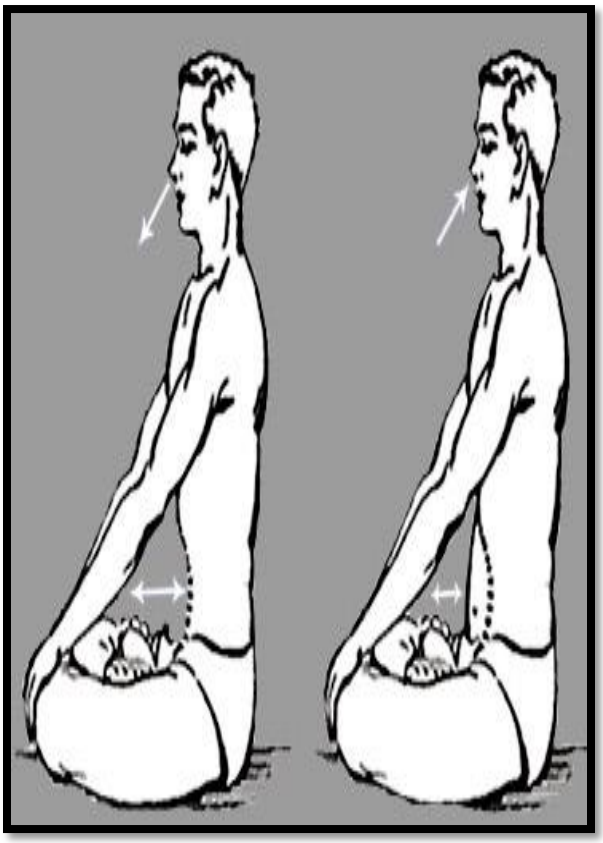


5.4.4 *Kapalbhati*, A *Yogic Kriya*:

bhasträvallohakärasya recapürau sasambhramau|
kapälabhätirviìkhyätä kaphadoñaviçoñaëé||

(Verse 35, Chapter 2, Hatha *Yoga* Pradipika)

Subjects were asked to perform forceful exhalation followed by passive inhalation through both nostrils at the rate of 1 cycle/second for the duration of 1 minute. This was one round and the same was repeated for 3 rounds with a gap of 1 minute between each round during which subjects were allowed to relax with normal breathing (rest period). Hence, the total duration including the rest period will be of 5 minutes.



The order of the sessions is provided in table 2.

Table 2: Order of the intervention session	
Number of orders	Orders of sessions
First (*n* = 5)	K, BA, BI, KA
Second (*n* = 5)	BA, BI, KA, K
Third (*n* = 5)	BI, KA, K, BA,
Fourth (*n* = 5)	KA, K, BA, BI
*K = *Kumbhaka*; BA = *Bhastrika*; BI = *Bhramari* and KA= *Kapalbhati*	

5.5 DATA EXTRACTION:

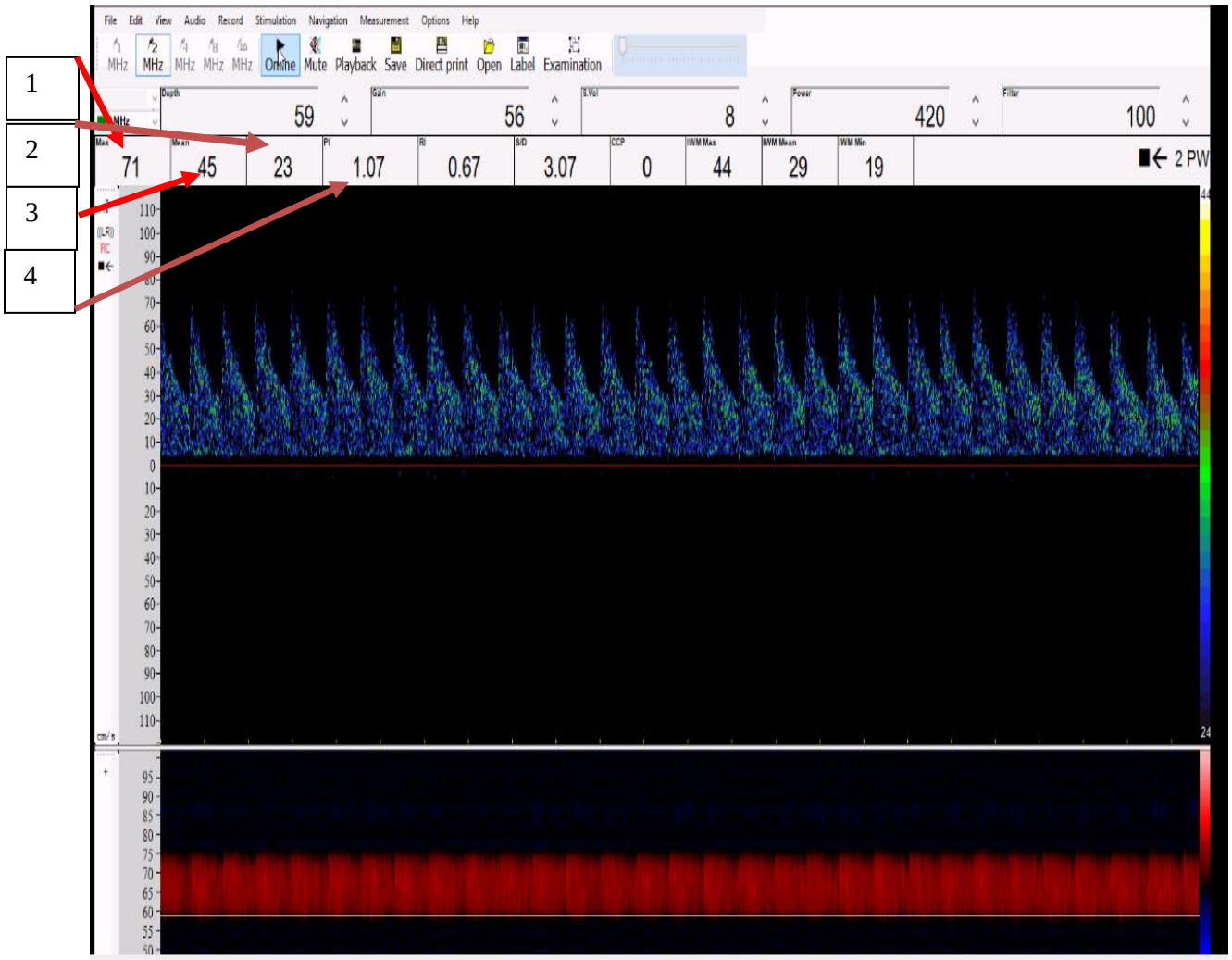
5.5.1 Cerebrovascular hemodynamic changes:

Based on the spectral patterns, cerebral hemodynamics in normal individuals was assessed. The normal spectral waveform shows a sharp systolic upstroke and stepwise deceleration with positive end-diastolic flow. The following data were extracted in offline TCD spectra:

1. *Peak systolic velocity (PSV in cm/s):* This is the first peak on a TCD waveform from each cardiac cycle.
2. *End-diastolic velocity (EDV in cm/s):* The end-diastolic flow velocity (EDV) lies between 20 and 50% of the peak systolic velocity (PSV) values, indicating a low resistance intracranial arterial flow pattern.
3. *Mean flow velocity (MFV in cm/s):* The mean flow velocity is calculated as EDV plus one-third of the difference between PSV and EDV.
4. *Pulsatility index (PI):* Flow resistance is usually assessed by PI, calculated by subtracting EDV from PSV and dividing the value by MFV. This is the most frequently used TCD

parameter to determine the flow resistance. PI is independent of the angle of insonation, has no unit, and a value more than 1.2 represents high resistance blood flow.

Figure 3: Raw Image of Transcranial doppler recording taken during the Baseline session



1= Peak systolic velocity (PSV in cm/s); 2= End-diastolic velocity (EDV in cm/s), 3= Mean flow velocity (MFV in cm/s), 4= Pulsatility index

5.5.2 Cardiovascular functions:

- The variables such as *Systolic Blood pressure, Diastolic Blood pressure, Mean Arterial Pressure, Stroke Volume, Left Ventricular Ejection Time, Pulse Interval, Total Cardiac Output, and Total Peripheral Resistance* were extracted in off-line and exported to Microsoft excel 2007.
- The data recorded was visually inspected off-line and only noise free data of *Heart rate* was included for analysis using the HRV analysis software.

Figure 4: Raw Image of ECG and respiration recording taken during Baseline session

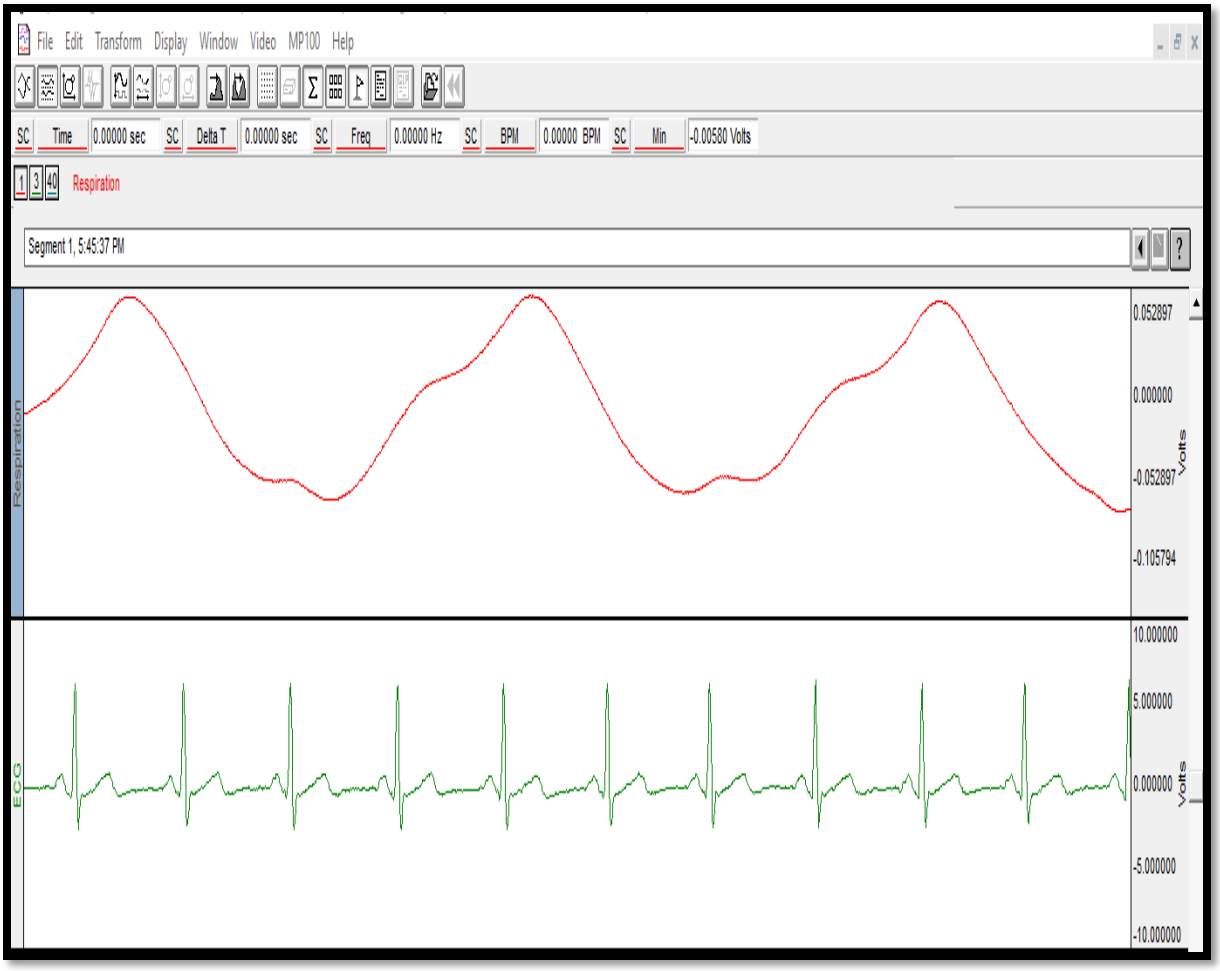
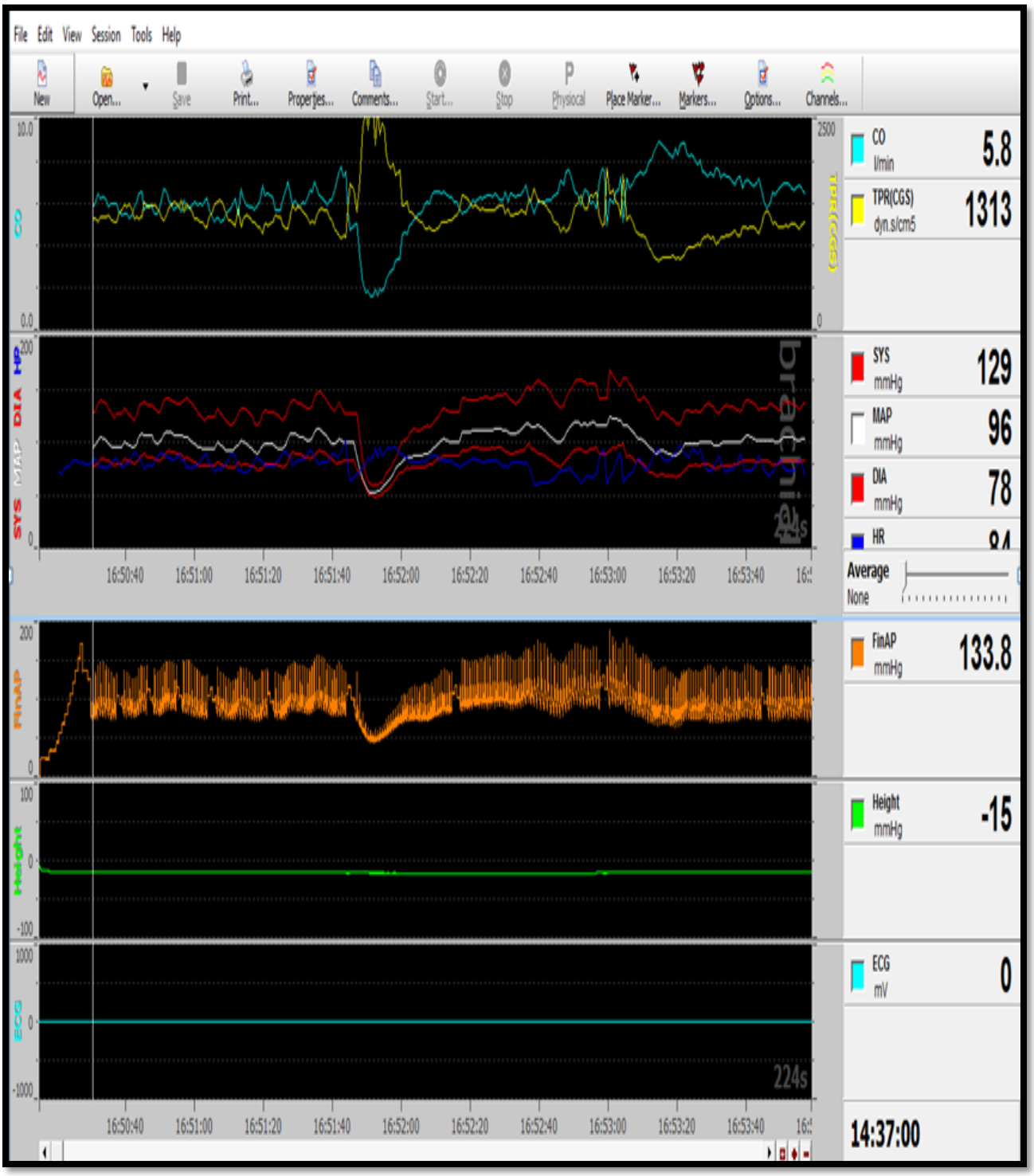


Figure 5: Raw Image of Cardiovascular parameters recording taken during Baseline session



Statistical Analysis:

Phase 1: Statistical analysis of cerebrovascular hemodynamics, Cardiovascular Functions and BHT between *Yoga* and non-*Yoga* practitioners was performed using "Independent samples-t-test" with the use of statistical package for the Social Sciences, version 16.

Phase 2: Statistical analysis of cerebrovascular hemodynamics and cardiovascular functions during various *Pranayama* techniques & a *Yogic Kriya* was performed using "Repeated measures of analysis of variance with post hoc analysis and Bonferroni adjustment" with the use of statistical package for the Social Sciences, version 16.p value <0.05 was considered as significant.