Original Article

Autonomic Response Latency Revisited: Assessment of Repeatability in Healthy Subjects

Annie S. Jose, Shival Srivastav, Bharati Mehta

Department of Physiology, All India Institute of Medical Sciences, Jodhpur, Rajasthan, India

Abstract

Background: Autonomic function assessment provides valuable information regarding the status of the autonomic nervous system. The time lag between the onset of orthostasis and the peak/nadir of heart rate response is a surrogate of the integrity of underlying autonomic neural pathways. Autonomic response latency (ARL) is a relatively novel yet underreported parameter in this context. Test-retest repeatability of this parameter has not been evaluated previously.

Materials and Methods: We recruited 31 healthy adults (17 males and 14 females; 29.00 ± 5.44 years) and subjected them to postural challenge tests on five instances – forenoon and afternoon of day 1, the next day, 1 week later, and 1 month later. Tachycardia and bradycardia latencies (TL and BL) were computed using heart rate derived from digital ECG data. Repeatability was assessed using the intraclass correlation coefficient (ICC) and coefficient of variation (CoV).

Results: ICCs for TL and BL were 0.69 (0.56, 0.82) and 0.77 (0.66, 0.87), respectively. The CoVs for TL and BL were 14.8% and 12.4%, respectively. Sex-based subgroup analysis revealed ICCs for TL and BL in males to be 0.71 (0.53, 0.86) and 0.74 (0.57, 0.88) and in females to be 0.68 (0.64, 0.86) and 0.82 (0.66, 0.93), respectively. CoVs for TL and BL were 14.4% and 13.8% in male subjects and 15.4% and 10.7% in female subjects, respectively.

Conclusion: ARL to orthostatic challenge demonstrated moderate to good test-retest repeatability. Based on our observations, we propose that ARL has potential as a consistent and repeatable index for the assessment of the integrity of autonomic neural pathways and therefore can help in the diagnosis of autonomic neuropathy.

Keywords: Autonomic denervation, autonomic nervous system, heart rate, neurophysiology

Address for correspondence: Dr. Shival Srivastav, Room C026, Department of Physiology, College Building, All India Institute of Medical Sciences, Basni Industrial Area Phase II, Jodhpur - 342005, Rajasthan, India.
E-mail: drshival@gmail.com
Submitted: 15-Oct-2023; **Revised:** 30-Jan-2024; **Accepted:** 31-Jan-2024; **Published:** 29-Jul-2024

Introduction

The autonomic nervous system (ANS) is essential to the regulation of various homeostatic processes in the human physiological system. The integrity of the ANS is routinely assessed using autonomic reactivity and cardiac autonomic tone.[1-3] Cardiac autonomic tone is assessed using heart rate variability (HRV), which involves computation of time domain and frequency domain indices to quantify resting autonomic tone.[4-6] Autonomic reactivity is quantified using a battery of tests proposed by Ewing *et al*.[7-10] This battery consists of physiological maneuvers such as orthostasis, deep breathing, Valsalva maneuver (VM), isometric exercise, and cold pressor challenge.

Latency is an important physiological parameter from a neurophysiological perspective. It represents the time lag between the stimulus and elicited response and is thus representative of the structural and functional integrity of the underlying neural pathway. It is commonly evaluated in

Access this article online

Quick Response Code:

Website: www.advbiores.net

DOI: 10.4103/abr.abr_409_23

How to cite this article: Jose AS, Srivastav S, Mehta B. Autonomic response latency revisited: Assessment of repeatability in healthy subjects. Adv Biomed Res 2024;13:60.

neurophysiological signals such as evoked responses. For ANS evaluation, latency is usually estimated in the context of sympathetic skin response (SSR). Increased latency is usually due to structural and/or functional alteration in the underlying neural pathways and symbolizes underlying pathology. Increased SSR latency has been attributed to subclinical obliteration of sympathetic pathways in disorders such as diabetes mellitus.[11,12] Therefore, the estimation of latency is likely to provide important information both in health and disease.

Autonomic response latency (ARL) represents physiological changes in heart rate (HR) in response to provocative maneuvers such as postural challenge tests such as lying to standing test (LST)/head-up tilt test and VM. Postural challenge leads to pooling of blood in the lower extremities and a fall in blood pressure. This is followed by compensatory changes in the heart mediated by the baroreflex.[13,14] In contrast, VM reduces preload to the heart due to increased intrathoracic pressure. This leads to characteristic phasic changes in blood pressure and HR – divided into phases I–IV – which are mediated through baroreflex and multiple other physiological mechanisms.[15-20] Both postural challenge and VM produce characteristic changes in HR that can be used to compute ARL. Normal ranges of ARL and the effect of physical training on this parameter have been reported elsewhere.[21]

Although there are reports of ARL recorded for small populations, there are no reports of the test-retest consistency of this parameter. Absence of such validation may be one of the reasons for the sparse use of this index as a robust laboratory marker of autonomic integrity. Therefore, we undertook the present work to assess the test-retest repeatability of ARL in apparently healthy subjects.

Materials and Methods

The study was cross-sectional and observational in design. We followed the STROBE guidelines for observational studies for this work.[22] Subject recruitment began after obtaining ethical clearance from the institute ethics committee at our center. The sample size was estimated using a previous study by Sharma *et al*.[21] Participant enrollment and data collection were performed between July 2021 and June 2023. Apparently healthy adults of either sex, aged 18–45 years, who were willing to provide written informed consent were invited to participate in the study.

Thirty-nine healthy subjects meeting inclusion criteria were initially recruited for the study. Five subjects were excluded due to loss of follow-up for future visits. One subject complained of discomfort during the LST test and withdrew from the study protocol. Two subjects were found to be hypertensive during repeated baseline blood pressure measurements in the laboratory and were thus excluded from the study. Therefore, data of 31 subjects (17 males and 14 females) were finally included in the study.

Subjects suffering from any chronic disease likely to affect the ANS or on medications for any disorders were excluded from the study. A detailed medical history was obtained from all study participants before their inclusion in the study. In addition, a screening of autonomic symptoms was performed using a questionnaire used at our laboratory. The questionnaire involves common autonomic symptoms such as dizziness, sweating disturbances, recurrent diarrhea/constipation, micturition disturbances, and intolerance to hot/cold temperatures.

Informed written consent was obtained from all study participants, and the study protocol was described in detail. Abstinence from tea/coffee was ensured on the day of the tests. In addition, the study participants were requested to refrain from heavy exercise 24 hours before the recordings.

Test-retest variability of physiological parameters can be ascribed to three common factors: individual, environmental, and experimenter variability.[23] Assessment of individual variability, if any, was the goal of the present work. The recordings were done in a noise-free, temperature- and humidity-controlled environment at the Autonomic Function Laboratory at our center to avoid variability due to environmental factors. All the recordings were performed by a single observer to avoid experimenter variability.

Post arrival to the lab, the weight and height of the subject were measured and BMI was computed. This was followed by application of adhesive disposable Ag-AgCl ECG electrodes in the Lead II configuration. Respiration was recorded using a digital stethograph tied around the 4th intercostal space. The subjects were requested to lie supine for 5 minutes. Data were acquired using the Bionomadix™ wireless module of the Biopac MP 150™ (Biopac Systems Inc., USA) system. The signal was acquired at 2 kHz and bandpass filtered with 0.5 and 35 Hz. HR and RR intervals (RRi) were derived from the filtered ECG signal in real time. Acqknowledge™ software version 4.4 (Biopac Systems Inc., USA) was used for the purpose of data acquisition, visualization, and analysis.[24]

After a supine rest of 5 minutes, LST was performed as per standard protocol. The subjects were requested to assume standing posture from supine posture within 3 seconds. Thereafter they remained standing for 5 minutes. Lead II ECG and respiration signals were acquired throughout the protocol.

Basal tachycardia and bradycardia latencies (BLs) were calculated as described previously.[21] The time lag from assumption of standing posture and peak HR was designated as tachycardia latency (TL). BL was computed as the time difference between assumption of standing posture and nadir of HR. These values were computed using the following workflow. A digital marker was placed in the data acquisition software window immediately upon assumption of standing posture, designated as t_0. Post completion of LST, the ECG data were selected from the assumption of standing posture (coinciding with the digital marker), and HR and inter-beat intervals were extracted from the selection. The

in-built ECG interval extraction toolbox of Acqknowledge™ software was used for this purpose. The values were exported to a spreadsheet program, Microsoft Excel 2021™ (Microsoft, Redmond, USA). The time to HR peak and HR nadir were computed from t_0 and designated as TL and BL, respectively.

These values computed on the forenoon of day 1 were designated as baseline (V1). The entire protocol was repeated in the afternoon (2 hours post lunch on day 1, V2), on the next day (day 2, V3), 1 week later (day 7, V4), and 1 month later (day 30, V5) after the baseline reading. All the recordings (V1 and V3–5) were performed in the forenoon (between 9 AM and 12 noon) for all subjects, except the afternoon reading on day 1 (V2, which was done between 3 pm and 5 pm). Both TL and BL were computed for all instances for all subjects.

The values were tabulated in a spreadsheet program. Gaussian fit of data was checked using Shapiro-Wilk test. Repeatability was checked using the intraclass correlation coefficient (ICC) and coefficient of variation (CoV). ICCs were calculated as a single rater type with a two-way random effect model, designated as ICC (2,1). MedCalc™ Statistical Software version 19.2.6 (MedCalc Software BV, Ostend, Belgium)[25,26] was used for statistical analysis.

Results

Thirty-one subjects (17 males and 14 females; mean age = 29.00 ± 5.44 years) participated in the study. Male and female subjects were age-matched. While the height and weight of male subjects were significantly higher than those of female subjects, they were comparable with respect to BMI. The values are summarized in Table 1.

Table 1: Study participant characteristics

	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m²)
All participants	29.00±5.44	164.29±10.19	65.42±14.21	24.03±3.40
Males (*n*=17)	29.12±5.54	171.41±6.67	71.82±13.60	24.29±3.25
Females (*n*=14)	28.86±5.52	155.64±6.15	57.64±10.92	23.71±3.68
P	0.97	0.001	0.003	0.64

Repeatability of tachycardia and bradycardia latencies

The median values of TL were 7.85 (6.52–8.33), 8.32 (6.65–9.59), 8.00 (7.21–8.72), 8.35 (6.86–9.24), and 8.50 (7.29–9.51) s for visits 1–5, respectively. BLs were 16.87 (14.42–18.67), 17.15 (14.68–19.16), 16.58 (14.83–21.47), 18.30 (15.51–22.27), and 17.01 (15.82–18.87) s for visits 1–5, respectively (Figures 1 and 2).

We assessed repeatability for both TL and BL by using the ICC and CoV. The ICCs for TL and BL were 0.69 (0.56–0.82) and 0.77 (0.66–0.87), respectively. The CoVs for TL and BL were 14.8% and 12.4%, respectively.

Subgroup analysis according to sex revealed that the ICCs for TL and BL for male subjects were 0.71 (0.53–0.86) and 0.74 (0.57–0.88), respectively. Similarly, the ICCs for TL and BL for female subjects were 0.68 (0.64–0.86) and 0.82 (0.66–0.93), respectively. The CoVs for TL and BL for male subjects were 14.4% and 13.8%, respectively. Similarly, the CoVs for TL and BL for female subjects were 15.4% and 10.7%, respectively.

Discussion

The ANS plays a key role in homeostasis through its two mutually antagonistic limbs – sympathetic and parasympathetic systems. The integrity of the system is routinely checked using HRV and Ewing's battery of tests.[27-29] In addition to these two modalities, discrete limbs of the ANS can be assessed using specialized tests such as SSR, pupillometry, and muscle sympathetic nerve activity.[30-32]

ARL is a relatively simple and novel parameter that provides information regarding the integrity of the underlying neural pathways. Increased latency is suggestive of damage to the

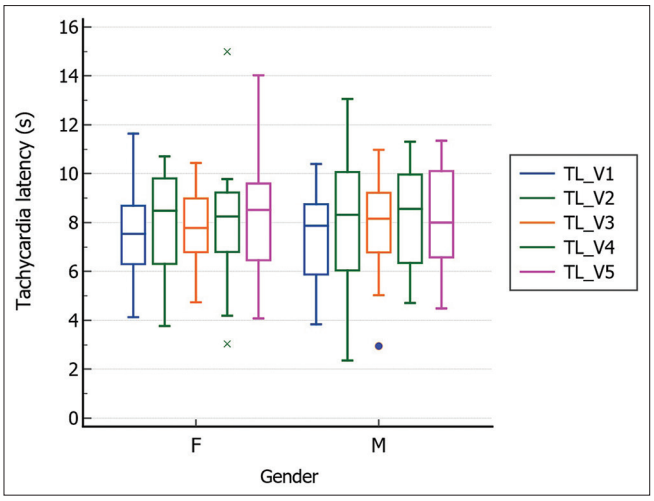

Figure 1: Tachycardia latencies for male and female subjects for five visits

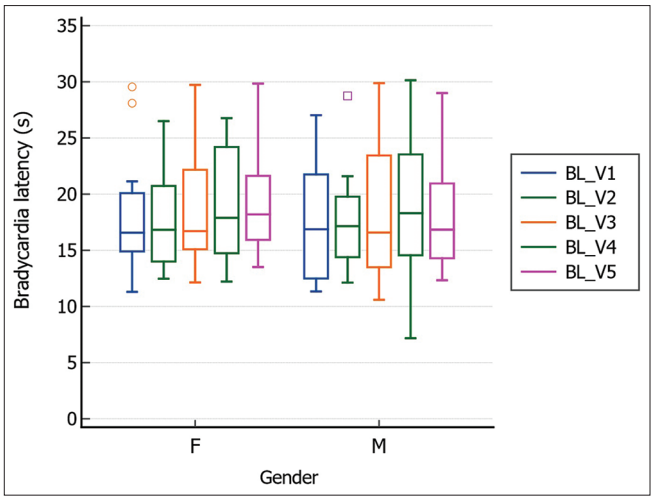

Figure 2: Bradycardia latencies for male and female subjects for five visits

reflex pathways, which may be structural and/or functional. It is assessed using HR response to physiological maneuvers such as postural challenge and VM. These maneuvers lead to perturbations in blood pressure, leading to reflex engagement of autonomic neural pathways and compensatory corrections. The HR response to postural challenge is characteristic. There is immediate tachycardia that is attributed to parasympathetic withdrawal. This change has been ascribed to multiple other mechanisms such as exercise pressor reflex, baroreflex, and central command.[33-36] The resultant increase in cardiac output is followed by baroreflex-mediated correction, leading to bradycardia around the 30th beat. This characteristic biphasic heart response to postural challenge is used to derive the 30:15 ratio, an important parasympathetic index.[37,38] The time to peak HR has been proposed to be approximately 5–12 s.[37] However, it is interesting to know the exact onset of peak tachycardia and bradycardia in response to orthostasis as it is a property of the underlying neural pathways and thus likely to show individual variation.

Another important consideration is the repeatability of ARL, which describes the robustness of the index. Our data are comparable with the values reported previously by Sharma *et al*. (TL: 6.44 ± 1.53 s; BL: 15.56 ± 3.34 s). As discussed previously, reports on repeated measurements of the indices in the same individual could not be found.

Test-retest reliability can be assessed by various measures such as the ICC and CoV.[23,39] We computed both the parameters in the present work. While there is no universally accepted classification of range of ICCs, a value between 0.5 and 0.75, 0.75 and 0.9, and those greater than 0.9 are suggestive of moderate, good, and excellent reliability, respectively.[40] In the present study, we observed moderate to good repeatability of TL and BL with reasonably low coefficients of variation. This is suggestive of the fact that response latency indices have the potential to serve as consistent and repeatable indices for assessment of integrity of autonomic neural pathways.

Previous literature compared TL and BL by using postural challenge and VM. We could not assess ARL by using VM in view of the COVID-19 pandemic as it was not possible to ensure complete sterilization of the sphygmomanometer apparatus used to gauge expiratory pressure during the maneuver.[41,42] Estimation of TL and BL using VM would have provided better information about ARL.

There are a few limitations to the study. A larger sample size across age groups would have provided better statistical confirmation of the results. In addition, the availability of continuous beat-to-beat blood pressure would have provided additional information regarding the latency of the baroreflex-mediated corrections.

Conclusion

To conclude, we propose that ARL may serve as a feasible measure of latency of the autonomic neural pathways as HR and corresponding RR intervals are relatively easier to record and measure when compared to complex responses such as SSR. Estimation of ARL parameters across age groups is essential to establish normative data. In addition, the exploration of these indices in disorders affecting the autonomic neural pathways will establish diagnostic validity, if any, in the future.

Data are expressed as median (interquartile range). F and M represent female and male subjects, respectively. TL represents tachycardia latency in seconds. V1–V5 represent visits 1–5, respectively. Outliers are shown as symbols for respective visits.

Data are expressed as median (interquartile range). F and M represent female and male subjects, respectively. BL represents bradycardia latency in seconds. V1–V5 represent visits 1–5, respectively. Outliers are shown as symbols for respective visits.

Values expressed as Mean ± SD. Unpaired *t*-test was used to compare the parameters between male and female subjects. While males had significantly higher height and weight as compared to their female counterparts, both groups were comparable with respect to body mass index (BMI).

Acknowledgements

The authors acknowledge the assistance provided by Mr. Shiv Kumar, laboratory staff, in the collection of data.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors do not declare any conflict of interest, financial or otherwise.

References

1. Tewari HK, Gadia R, Kumar D, Venkatesh P, Garg SP. Sympathetic-parasympathetic activity and reactivity in central serous chorioretinopathy: A case-control study. Invest Ophthalmol Vis Sci 2006;47:3474-8.
2. Jyotsna VP, Singh AK, Deepak KK, Sreenivas V. Progression of cardiac autonomic dysfunction in newly detected type 2 diabetes. Diabetes Res Clin Pract 2010;90:e5-6.
3. Khandelwal E, Jaryal AK, Deepak KK. Pattern and prevalence of cardiovascular autonomic neuropathy in diabetics visiting a tertiary care referral center in India. Indian J Physiol Pharmacol 2011;55:119-27.
4. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task force of the European Society of cardiology and the North American society of pacing and electrophysiology. Eur Heart J 1996;17:354-81.
5. Pham T, Lau ZJ, Chen SHA, Makowski D. Heart rate variability in psychology: A review of HRV indices and an analysis tutorial. Sensors (Basel) 2021;21:3998.
6. Santos-de-Araújo AD, Shida-Marinho R, Pontes-Silva A. Heart rate variability (HRV): Checklist for observational and experimental studies. Autoimmun Rev 2022;21:103190.
7. Ewing DJ, Campbell IW, Murray A, Neilson JM, Clarke BF. Immediate heart-rate response to standing: Simple test for autonomic neuropathy in diabetes. Br Med J 1978;1:145-7.
8. Ewing DJ. Testing for autonomic neuropathy. Lancet 1981;1:224.
9. Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic

neuropathy. Br Med J (Clin Res Ed) 1982;285:916-8.
10. Ewing DJ, Martyn CN, Young RJ, Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. Diabetes Care 1985;8:491-8.
11. Ueda T, Yoshimura N, Yoshida O. Diabetic cystopathy: Relationship to autonomic neuropathy detected by sympathetic skin response. J Urol 1997;157:580-4.
12. Nazhel B, Yetkin I, Irkeç C, Koçer B. Sympathetic skin response in diabetic neuropathy. Electromyogr Clin Neurophysiol 2002;42:181-5.
13. van Lieshout JJ, Harms MP, Pott F, Jenstrup M, Secher NH. Stroke volume of the heart and thoracic fluid content during head-up and head-down tilt in humans. Acta Anaesthesiol Scand 2005;49:1287-92.
14. Laitinen T, Niskanen L, Geelen G, Länsimies E, Hartikainen J. Age dependency of cardiovascular autonomic responses to head-up tilt in healthy subjects. J Appl Physiol (1985) 2004;96:2333-40.
15. Hiner BC. Valsalva maneuver. Clin Med Res 2005;3:55.
16. Looga R. The Valsalva manoeuvre--cardiovascular effects and performance technique: A critical review. Respir Physiol Neurobiol 2005;147:39-49.
17. Pstras L, Thomaseth K, Waniewski J, Balzani I, Bellavere F. The Valsalva manoeuvre: Physiology and clinical examples. Acta Physiol (Oxf) 2016;217:103-19.
18. Srivastav S, Jamil RT, Zeltser R. Valsalva maneuver. In: StatPearls. StatPearls publishing: Treasure island (FL), 2023 http://www.ncbi.nlm.nih.gov/books/NBK537248/. [Last accessed on 3 Jun 2023].
19. Goldstein DS, Cheshire WP Jr. Beat-to-beat blood pressure and heart rate responses to the Valsalva maneuver. Clin Auton Res 2017;27:361-7.
20. Srivastav S, Chandran DS, Jaryal AK, Deepak KK. Comparison of baroreflex responses to lower body negative pressure and Valsalva maneuver in healthy subjects. Indian Journal of Physiology and Pharmacology 2018;62:278-85.
21. Sharma RK, Deepak KK, Bijlani RL, Rao PS. Short-term physical training alters cardiovascular autonomic response amplitude and latencies. Indian J Physiol Pharmacol 2004;48:165-73.
22. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, *et al*. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: Guidelines for reporting observational studies. J Clin Epidemiol 2008;61:344-9.
23. Bartlett JW, Frost C. Reliability, repeatability and reproducibility: Analysis of measurement errors in continuous variables. Ultrasound Obstet Gynecol 2008;31:466-75.
24. Ravichandran S, Srivastav S, Kamble PH, Chambial S, Shukla R, Sharma P, *et al*. VEGF-A and cardiac autonomic function in newly diagnosed type 2 diabetes mellitus: A cross-sectional study at a tertiary care center. J Family Med Prim Care 2019;8:3185-90.
25. Schoonjans F, Zalata A, Depuydt CE, Comhaire FH. MedCalc: A new computer program for medical statistics. Comput Methods Programs Biomed 1995;48:257-62.
26. Schoonjans F, Zalata A, Depuydt CE, Comhaire FH. MedCalc: a new computer program for medical statistics. Comput Methods Programs Biomed. 1995;48:257-62.
27. Tomasi J, Zai CC, Pouget JG, Tiwari AK, Kennedy JL. Heart rate variability: Evaluating a potential biomarker of anxiety disorders. Psychophysiology 2024;61:e14481.
28. Roche F, Pichot V, Mouhli-Gasmi L, Monier M, Barthélémy JC, Berger M, Celle S, Chouchou F. Anatomy and physiology of the autonomic nervous system: Implication on the choice of diagnostic/monitoring tools in 2023. Rev Neurol (Paris). 2024;180:42-52.
29. Prokhorov MD, Karavaev AS, Ishbulatov YM, Ponomarenko VI, Kiselev AR, Kurths J. Interbeat interval variability versus frequency modulation of heart rate. Phys Rev E 2021;103:042404.
30. Ravits JM. AAEM minimonograph #48: Autonomic nervous system testing. Muscle Nerve 1997;20:919-37.
31. Low PA. Testing the autonomic nervous system. Semin Neurol 2003;23:407-21.
32. Low PA. Laboratory evaluation of autonomic function. Suppl Clin Neurophysiol 2004;57:358-68.
33. Borst C, Wieling W, van Brederode JF, Hond A, de Rijk LG, Dunning AJ. Mechanisms of initial heart rate response to postural change. Am J Physiol 1982;243:H676-81.
34. Borst C, van Brederode JF, Wieling W, van Montfrans GA, Dunning AJ. Mechanisms of initial blood pressure response to postural change. Clin Sci (Lond) 1984;67:321-7.
35. Wieling W, Borst C, Karemaker JM, Dunning AJ. Testing for autonomic neuropathy: Initial heart rate response to active and passive changes of posture. Clin Physiol 1985;5(Suppl 5):23-7.
36. Wieling W, Borst C, van Brederode JF, van Dongen Torman MA, van Montfrans GA, Dunning AJ. Testing for autonomic neuropathy: Heart rate changes after orthostatic manoeuvres and static muscle contractions. Clin Sci (Lond) 1983;64:581-6.
37. Freeman R. Assessment of cardiovascular autonomic function. Clin Neurophysiol 2006;117:716-30.
38. Freeman R, Chapleau MW. Testing the autonomic nervous system. Handb Clin Neurol 2013;115:115-36.
39. Bartko JJ. The intraclass correlation coefficient as a measure of reliability. Psychol Rep 1966;19:3-11.
40. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med 2016;15:155-63.
41. Figueroa JJ, Cheshire WP, Claydon VE, Norcliffe-Kaufmann L, Peltier A, Singer W, *et al*. Autonomic function testing in the COVID-19 pandemic: An American autonomic society position statement. Clin Auton Res 2020;30:295-7.
42. Arvind A, Srivastav S. Cardiac autonomic function evaluation during the COVID-19 pandemic: A frugal innovation for Valsalva manoeuvre. Indian Journal of Physiology and Pharmacology 2021;65:66-8.