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Comparison of Autonomic Indices in Normal Weight, Overweight and Obese Adults – a Comparative Analysis Using South East Asian Criteria for Obesity Classification

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ABSTRACT

Objectives: *Autonomic function assessment, performed using heart rate variability and Ewing's battery, can help unravel autonomic neuropathy in many clinical disorders. This assessment can be affected by obesity. We revisited the relationship between autonomic function parameters and obesity. The body mass index (BMI) based stratification based on standard World Health Organization (WHO) classification and revised South East Asian (SEA) classification was employed.*

Materials and methods: *Apparently, healthy subjects of either sex were included in the present study. Subjects were classified into normal weight (NOR), overweight (OW) and obese (OB) categories using both guidelines. An autonomic function test was performed, documenting heart rate variability (HRV) and Ewing's battery of tests including postural challenge, deep breathing, cold pressor and isometric exercise. Parameters were compared between the three groups.*

Results: *We recruited 105 healthy subjects (89 males, 16 females) with a mean age of 29.83 ± 5.44 years. There were 33, 29 and 43 subjects in the NOR, OW and OB categories, respectively, as per the revised SEA classification. Similarly, 63 subjects were NOR, 42 OW, and six OB, as per the WHO criteria. There was no significant difference between HRV parameters and autonomic reactivity parameters between NOR, OW and OB subjects as per the revised SEA and WHO criteria.*

Conclusions: *The WHO criteria underestimated obesity in our population sample. In addition, there was no significant difference in autonomic parameters between NW, OW and OB subjects as per the SEA criteria. We propose that the effect of obesity on autonomic parameters may require a revisit in light of revised BMI criteria for the SEA population.*

Keywords: autonomic neuropathy, body mass index, heart rate variability, obesity.

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INTRODUCTION

The autonomic nervous system (ANS) orchestrates multiple physiological mechanisms across diverse organ systems. The mutually antagonistic limbs of ANS – sympathetic and parasympathetic – are vital for maintaining homeostasis. Autonomic neuropathy is a hallmark of multiple clinical disorders such as diabetes mellitus (1), Parkinson's disease (2, 3) and multisystem atrophy (4, 5). Therefore, laboratory evaluation of the ANS can provide vital clues essential for the timely diagnosis and management of such disorders.

Laboratory assessment is performed using an array of methods designed to evaluate the sympathetic and parasympathetic nervous systems. Since ANS supplies almost all organs in the body, a comprehensive evaluation requires testing for different organ systems using a gamut of techniques. Heart rate variability (HRV) and a battery of tests proposed by Ewing and colleagues (6, 7) are the cornerstones for diagnosing cardiac autonomic neuropathy (CAN), suggestive of the affliction of the cardiovascular limb of ANS. Other novel techniques have also been proposed and evaluated as adjuncts for comprehensive CAN assessment. These include baroreflex sensitivity (BRS) (8), blood pressure variability (BPV) (9), muscle sympathetic nerve activity (MSNA) (10) and autonomic response latency (ARL) (11, 12).

Ewing's battery of tests includes simple physiological maneuvres such as deep breathing, orthostasis, Valsalva maneuver, cold exposure and isometric exercise. The blood pressure and/or heart rate responses to these maneuvres are compared with published cut-offs to grade autonomic neuropathy using classification methods proposed by Ewing and colleagues. Another popular valuable system for CAN grading is Bellavere's scoring system (13, 14). Heart rate variability and Ewing's battery provide a non-invasive and simple method for CAN assessment with excellent patient acceptability. Also, these tests provide a comprehensive picture of sympathovagal influence on the cardiovascular system. Therefore, they are widely used in physiology laboratories around the globe for CAN screening and reporting.

Obesity is a problem of global magnitude, affecting one in every eight persons in 2022, *as per* WHO (15). Also, it has been noticed that obesity in early life can lead to a myriad of cardiometabolic diseases in the later part of life (16-18). The body mass index (BMI) is a common index used for the classification of obesity, wherein BMI ≥ 30 kg/m^2 is designated as obese (19). However, this classification system is being challenged to account for variation according to different population subgroups based on geographical regions and ethnicities. A classification for the Southeast Asian population was proposed a few years ago (20), which reduced the cut-off of BMI for the Asian population. Along these lines, a consensus statement was issued reducing the BMI cut-offs for obesity for the Indian population. According to this classification system, a BMI of 23–24.9 kg/m^2 is defined as overweight and a BMI ≥25 kg/m^2 is labelled as obese (21, 22).

Research has consistently shown that obesity is a significant factor affecting the ANS. It has been proposed that obesity predisposes to sympathetic overactivity and, therefore, can lead to hypertension and other cardiovascular issues (23-25). However, the majority of studies have employed the WHO-based classification of obesity, which is based on BMI, for such assessments. We believed it might be interesting to assess autonomic indices in healthy subjects when stratified by the recently proposed Asian classification for obesity. In this context, we could find very few studies (26) that have attempted to explore this premise. Therefore, the present study was conducted to compare autonomic indices in normal-weight, overweight and obese adults and to explore any differences within these groups in our population. ❑

MATERIALS AND METHODS

We designed a cross-sectional and observational study. The protocol was approved by the Institute Ethics Committee (IEC) of our hospital and the study was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2000. We recruited apparently healthy adults of either sex who were free from any cardiovascular, neurological, or other diseases likely to affect the ANS. The subjects were non-smokers and did not have any history suggestive of substance addiction.

Upon obtaining written informed consent, subjects were requested to report to the Autonomic Function Laboratory of our department two hours after a light meal. Abstinence from tea/coffee was ensured on the day of the test. Also, participants were requested to refrain from heavy exercise or strenuous physical activity in the preceding 24 hours before autonomic evaluation. Female patients were requested to report for evaluation between days 2 and 6 of the menstrual cycle.

Participants' height was measured to the nearest centimeter using a wall-mounted stadiometer. Their weight was taken using an electronic weighing machine and was rounded off to the nearest kilogram. The BMI was calculated using Quetlet's index: weight (kg)/square of height (m^2). Subjects were categorized as normal weight (NOR), overweight (OW) or obese (OB) groups based on the revised SEA BMI criteria. A BMI of ≤ 22.9 kg/m^2 was considered NOR, 23–24.9 kg/m^2 was defined as OW and a BMI ≥25 kg/m^2 was labelled as OB. Conventional WHO criteria were also used to categorize subjects into NOR, OW and OB groups.

Participants were requested to void their bladders before the tests. Baseline blood pressure was taken after five minutes of supine rest using Omron™ BP monitor (model HEMCR24™). This was followed by the placement of adhesive ECG electrodes in Lead II configuration and a digital stethograph belt around the fourth intercostal space on the chest. BioNomandix™ module of Biopac MP 150™ system (Biopac Inc. USA) was used to acquire Lead II ECG and respiratory movement signals. Real-time computation and visualization of heart rate and RR intervals were done using derived channels. A 10-minute supine rest was provided, followed by five minutes of ECG acquisition for HRV analysis.

Autonomic reactivity was assessed by Ewing's battery of tests, which included a lying-to-standing test, a deep breathing test and an isometric exercise test, with an additional Cold Pressor test performed according to the AIIMS New Delhi protocol (27, 28). The Valsalva maneuver could not be performed as part of the study, which coincided with the COVID-19 pandemic, and it was not possible to ensure the sterility of the equipment for this test (29). The different maneuvers, which were performed as part of this battery, have been standardized according to the literature and are used in the Autonomic Function Laboratory of our hospital for routine testing (30, 31). All data was captured using Acqknowledge™ software version 4.4 (Biopac Inc. USA) and files were saved for offline analysis later.

The HRV analysis was performed using LabChart Pro™ software version 8 (AD Instruments, Australia). The time domain, frequency domain and non-linear indices were computed. SDNN, RMSSD, SDSD and pNN50% were chosen in the time domain. Total Power, LF-power, HF-power and LF/HF ratio were chosen in the frequency domain. The SD1, SD2 and SD1/SD2 ratios were chosen as indices for non-linear measures. All the aforementioned parameters were chosen as described in Task Force guidelines (32) and are well established in the literature (33-36).

For reactivity testing, the changes in systolic blood pressure (ΔSBP_LST), 30:15 ratio for lying to standing test, changes in heart rate (ΔHR_DBT), E:I ratio for deep breathing test, change in diastolic blood pressure after hand grip test (ΔDBP_HGT), and cold stimulation (ΔDBP_CPT), as described previously (30, 31) were calculated.

Values were tabulated in a spreadsheet program. Statistical analysis was performed using MedCalc software (MedCalc® Statistical Software version 23.1.3 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2025) (37). Shapiro Wilk test was used for the assessment of the Gaussian fit of parameters. Values were expressed as mean ± SD or median (interquartile range). Values were compared using the Kruskal Wallis test with the post-hoc Dunn's test. A p-value of < 0.05 was considered significant. ❑

RESULTS

A total of 105 subjects participated in the study (89 males, 16 females; mean age 29.83 ± 5.44 years). Upon classifying the subjects based on South East Asian criteria for obesity, Normal (NOR), Overweight (OW) and Obese (OB) groups consisted of 33, 29 and 43 subjects, respectively. There were 63 NOR, 36 OW and

TABLE 1. Anthropometric characteristics of study subjects

SNo.	Parameter	Value
1	Age	29.83 ± 5.44 years
2	Height	1.70 ± 0.08 m
3	Weight	70.59 ± 9.596 kg
4	Body mass index (BMI)	24.51 ± 3.04 kg/m^2

Values expressed as mean ± SD

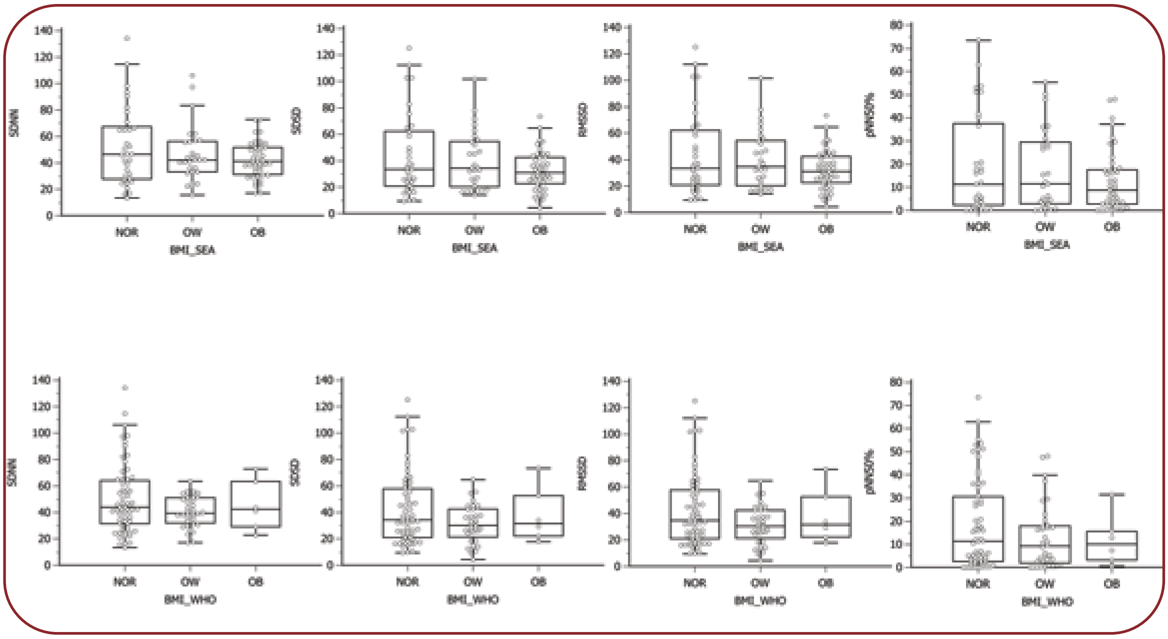

FIGURE 1. Comparison of time domain indices using WHO and SEA criteria for obesity. Values are represented as box and whisker plots. Individual data points are shown as hollow circles. There was no significant difference between parameters. _SEA and _WHO represent criteria followed for obesity classification. NOR, OW and OB represent 'Normal', 'Overweight' and 'Obese' as per the aforementioned criteria. SDNN=standard deviation of NN intervals; RMSSD=root mean square of standard deviation; SDSD=standard deviation of differences between NN intervals; pNN50%=percentage of NN intervals varying by more than 50 ms with respect to previous intervals

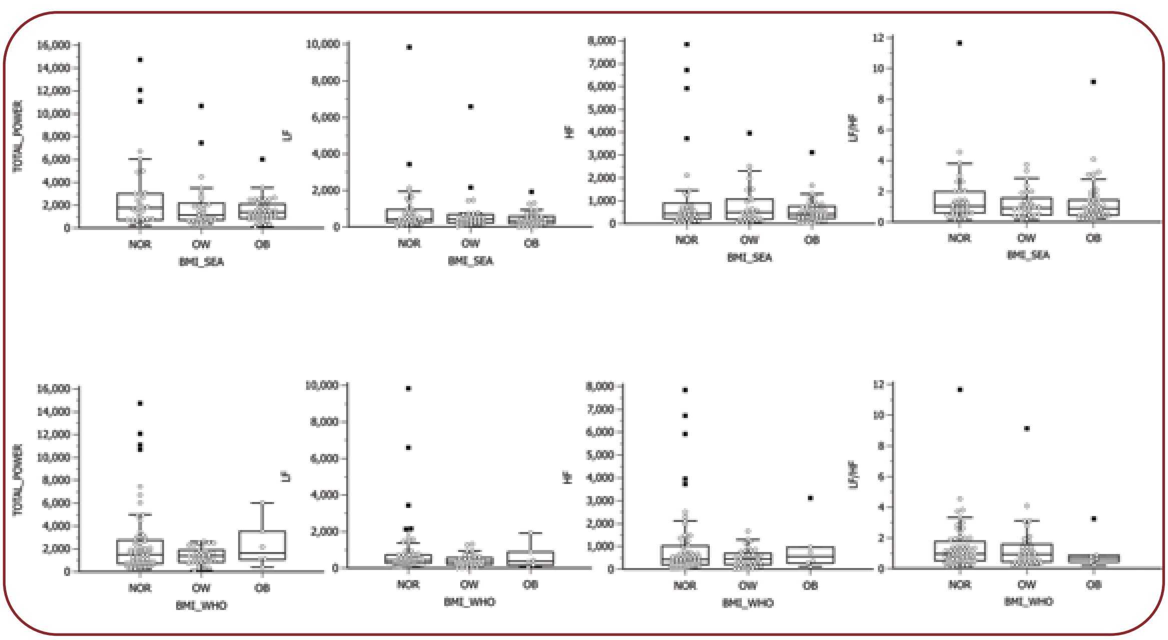

FIGURE 2. Comparison of frequency domain indices using WHO and SEA criteria for obesity. Values are represented as box and whisker plots. Individual data points are shown as hollow circles. Solid squares represent outliers. There was no significant difference between parameters. _SEA and _WHO represent criteria followed for obesity classification. NOR, OW and OB represent 'Normal', 'Overweight' and 'Obese' as per the aforementioned criteria. Total_Power: the overall variance of all NN intervals in the five-minute segment; LF: power in the low frequency (0.04-0.15 Hz) band; HF: power in the high frequency (0.15-0.4 Hz) band; LF/HF: the ratio of LF power and HF power

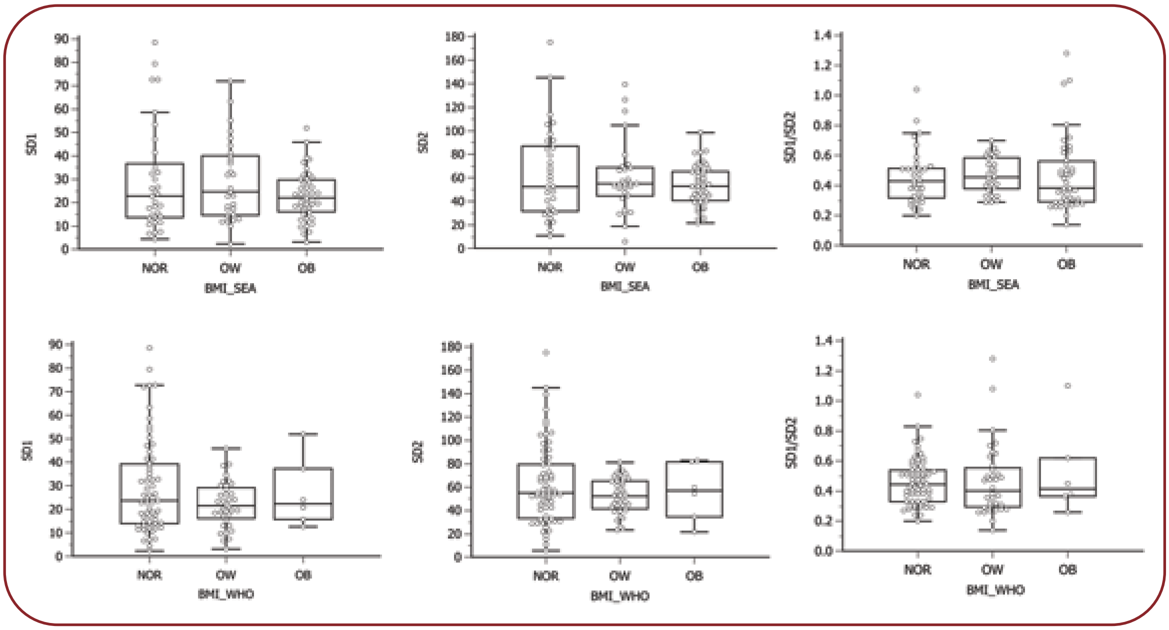

FIGURE 3. Comparison of non-linear indices using WHO and SEA criteria for obesity. Values are represented as box and whisker plots. Individual data points are shown as hollow circles. There was no significant difference between the parameters. _SEA and _WHO represent criteria followed for obesity classification. NOR, OW and OB represent 'Normal', 'Overweight' and 'Obese' as per the aforementioned criteria. SD1: standard deviation perpendicular to the line of identity; SD2: standard deviation along the line of identity; SD1/SD2: ratio of SD1 and SD2

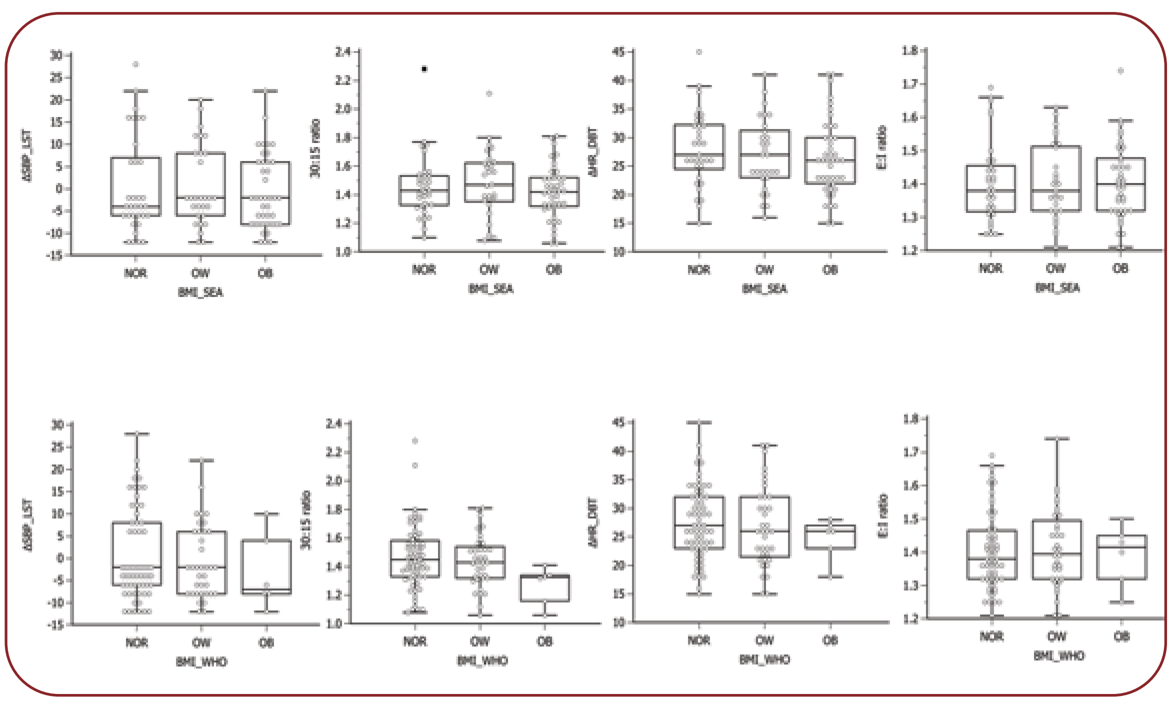

FIGURE 4. Comparison of reactivity indices using WHO and SEA criteria for obesity – Part 1. Values are represented as box and whisker plots. Individual data points are shown as hollow circles. There was no significant difference between the parameters. _SEA and _WHO represent criteria followed for obesity classification. NOR, OW and OB represent 'Normal', 'Overweight' and 'Obese' *as per* the aforementioned criteria. ΔSBP_LST: fall in systolic blood pressure after orthostatic challenge; 30:15 ratio: the ratio of RR intervals around the 30^{th} and 15^{th} beat after orthostasis; ΔHR_DBT: the difference in maximum and minimum heart rates averaged over six cycles in deep breathing test; and E:I ratio: the ratio of maximum and minimum RR intervals averaged over six cycles in deep breathing test

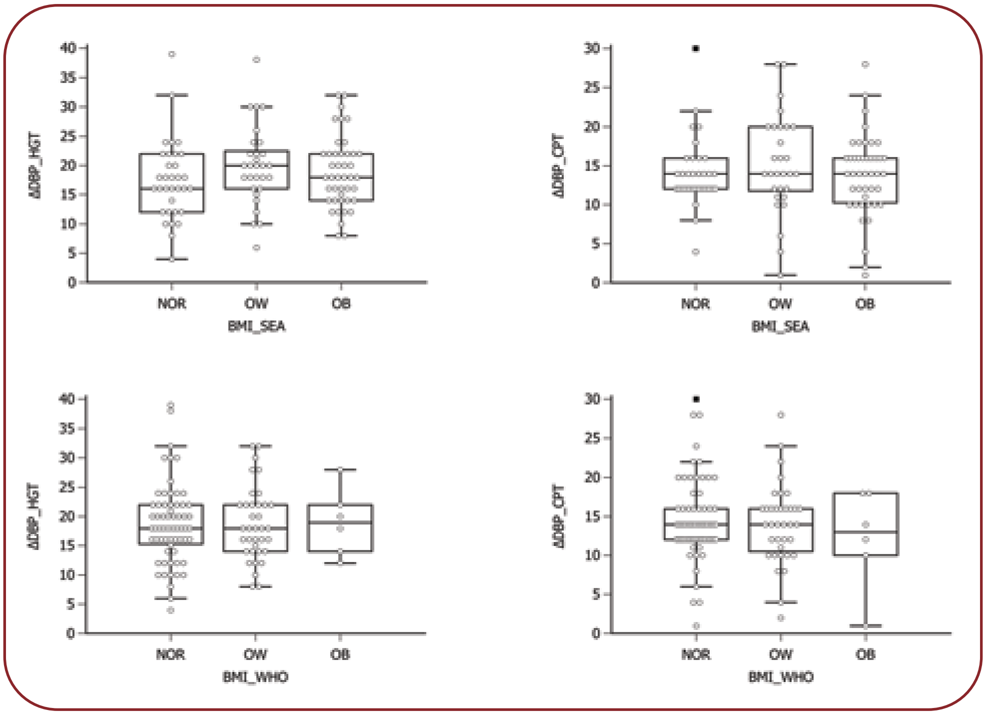

FIGURE 5. Comparison of reactivity indices using WHO and SEA criteria for obesity – Part 2. Values are represented as box and whisker plots. Individual data points are shown as hollow circles. There was no significant difference between the parameters. _SEA and _WHO represent criteria followed for obesity classification. NOR, OW and OB represent 'Normal', 'Overweight' and 'Obese' *as per* the aforementioned criteria. ΔDBP_HGT: rise in diastolic blood pressure after hand grip test; and ΔDBP_CPT: rise in diastolic blood pressure after cold pressor test

six OB subjects, *as per* WHO criteria. The anthropometric characteristics of study subjects are summarised in Table 1.

The indices for cardiac autonomic tone encompassed the time domain, frequency domain, and non-linear index. In the time domain, the standard deviation of NN intervals (SDNN), the root mean square of standard deviation (RMSSD), the standard deviation of differences between adjacent NN intervals (SDSD) and the percentage of NN intervals varying by more than 50 ms with respect to previous intervals (PNN50%) were analyzed. Median values for these parameters were 42.20 (31.21–55.58) ms, 33.12 (21.195–47.398) ms, 33.16 (21.23–47.46) ms and 10.48 (2.74–26.78) %, respectively. Frequency domain parameters that were computed included total power, high-frequency (HF) power, low-frequency (LF) power and LF/HF ratio. Median values for these indices were 1421.00 (792.93–2454.50) ms^2, 440.27 (213.59–815.45) ms^2, 389.71 (238.18–720.51) ms^2 and 0.92 (0.496–1.53), respectively. In non-linear measures, SD1, SD2 and SD1/SD2 were computed and their median values were 23.02 (14.55–32.98), 53.80 (39.40–70.04) and 0.44 (0.32–0.55), respectively.

For autonomic reactivity assessment, the lying to standing test, deep breathing test, hand grip (isometric exercise) test and cold pressor test were performed. ΔSBP_LST and 30:15 ratio indices were computed upon administration of lying to standing test and their median values were -2.00 (-8.00–6.00) mm Hg and 1.43 (1.32–1.56), respectively. For the deep breathing test, ΔHR_DBT and E:I ratio were calculated, and values were found to be 26.00 (23.00–32.00) beats/minute and 1.39 (1.32–1.47), respectively. Diastolic blood pressure change (ΔDBP) was calculated for the hand grip (isometric exercise) test and cold pressor test, and values were 18.00 (14.00–22.00) and 14.00 (12.00–16.00) mm Hg, respectively.

After the classification of participants into three categories (normal weight, overweight and obese) based on WHO and Southeast Asian criteria for obesity, the autonomic indices were compared across these three groups. There was no significant difference between SDNN, RMSSD, SDSD and pNN50% ($p= 0.42, 0.34, 0.34$ and 0.68 *as per* WHO criteria; and 0.54, 0.46, 0.46, and 0.67 *as per* SEA criteria, respectively, Kruskal Wallis test with Dunn's post hoc test, Figure 1). Similarly, no significant difference was found between total power, LF power, HF, power and LF/HF ratio ($p=0.589$, 0.42, 0.65 and 0.52 *as per* WHO criteria; and 0.53, 0.28, 0.82 and 0.56 *as per* SEA criteria, respectively, Kruskal Wallis test with post hoc Dunn's test, Figure 2). Geometric measures – SD1, SD2 and SD1/SD2 ratio – were also comparable between the three groups ($p= 0.55$, 0.61 and 0.73 *as per* WHO criteria; and 0.59, 0.82 and 0.46 *as per* SEA criteria respectively, Kruskal Wallis test with post hoc Dunn's test, Figure 3). Similar trends were noted in autonomic reactivity indices. ΔHR_DBT and E:I ratio, ΔSBP_LST and 30:15 ratio, ΔDBP_HGT and ΔDBP_CPT were found to be comparable between the study groups ($p= 0.52$, 0.06, 0.58, 0.95, 0.986, 0.79 *as per* WHO criteria; and 0.59, 0.43, 0.496, 0.99, 0.28 and 0.57 *as per* SEA criteria, respectively, Kruskal Wallis test with post hoc Dunn's test, Figures 4 and 5, respectively). ❑

DISCUSSION

In the present work, we compared autonomic indices, derived from heart rate variability and Ewing's battery of tests, in apparently healthy adults stratified into normal (NOR), overweight (OW) and obese (OB) categories using modified criteria for the Southeast Asian population. We observed that the application of universal WHO BMI-based criteria for obesity leads to an underestimation of obesity in our population. Therefore, it is prudent to apply SEA criteria for obesity classification in the population from our subcontinent to obtain an accurate estimate of the prevalence of obesity. In addition, we also observed that there was no significant difference in the autonomic parameters between NOR, OW, and OB subjects when classified according to SEA criteria. Similar observations were made when the WHO classification system was used.

For a comprehensive assessment of ANS, a combination of HRV and Ewing's battery of tests is widely employed. These tests are non-invasive, standardized, reproducible, employ physiological maneuvers, and have been shown to demonstrate good patient compliance. Ewing and colleagues described autonomic reactivity tests in their seminal work almost four decades ago. The cut-offs described in this work have laid the foundation for cut-offs for CAN assessment and have been rigorously validated in multiple studies. Cold pressor test was not part of the initial battery but was added later and is now included as a part of Ewing's battery. Also, the rise in diastolic blood pressure of 16 mm Hg in response to handgrip exercise was modified and reduced to 10 mm Hg. We currently follow the AIIMS New Delhi criteria (28) in our center, which report a diastolic blood pressure response of 10 mm Hg for both the Cold Pressor test and the handgrip test. Additionally, we assessed cardiac autonomic tone using HRV. We have recently evaluated the repeatability of these tests and have observed good repeatability for most of the indices (31). Therefore, these tests remain among the first-line tests used for CAN assessment.

Normative data for autonomic indices has been explored by multiple groups for our country's population. Sharma *et al* have described normative data on adolescents from the southern part of our country (38). In contrast, Shaikh *et al* have described normative data on the adolescent population from the state of Gujarat (39). There are also reports of normative data on autonomic indices in the adult population. Rani *et al* have documented HRV indices in over 370 adults from north India (40). In addition, Kunikullaya and colleagues have documented normative data in adults aged 18-30 years from the South Indian population (41). Since obesity is a significant problem and can affect autonomic measures, there is a need for further exploration of any relationship between these two entities.

There are multiple reports of ANS being affected by obesity, as discussed previously. However, there is disagreement regarding the nature and extent of sympathovagal modulation in this condition. Sympathetic underactivity, as well as overactivity, have been proposed in obese subjects, resulting in a lack of consensus (42). We observed that the majority of studies in this domain have employed the WHO criteria for classifying study subjects as normal weight, overweight, or obese. The same approach has also been followed in some studies from the Indian subcontinent (43, 44). In light of the new classification scale, it may be prudent to use the revised system for obesity stratification for our population. We found one study (26) in which the authors used modified classification criteria for subjects from the southern part of our country. They did not observe any significant difference between the groups in sympathetic and parasympathetic indices. However, the authors did not assess autonomic tone and only evaluated reactivity indices. In the present work, we evaluated a larger sample, classifying subjects into three discrete categories – NOR, OW and OB. Additionally, we evaluated autonomic tone markers in conjunction with reactivity tests to enhance the comprehensiveness of our evaluation. We did not observe any significant difference between the indices in the three groups. Based on our findings, we can infer that the WHO classification for obesity may not be suitable for the Indian population, as it underestimates the magnitude of obesity in our population. We may infer that there is a need for stratification using modified BMI-based criteria for the East Asian and Indian populations. In addition, a comprehensive classification using other anthropometric indices, in conjunction with BMI, may provide a more holistic picture of obesity classification.

Interestingly, we did not observe any difference in autonomic tone as well as autonomic reactivity indices between NOR, OW and OB subjects. Our findings are partially consistent with previous work (26). This opens an interesting avenue for further exploration into the effect of BMI on ANS function since it is in contradiction to the purported relationship between obesity and ANS.

There are a few limitations to our current work. Although we were able to draw meaningful inferences regarding the effect of obesity on autonomic indices using SEA criteria, similar conclusions could not be drawn when applying WHO criteria. This was because the OB group, *as per* WHO classification, consisted of only six subjects. This was beyond our control, as our primary aim was to assess the impact of SEA criteria. Additionally, our study population consisted of fewer female subjects (n=16), which prevented us from commenting on the effect of sex on differences in autonomic indices among normal-weight, overweight and obese subjects. Multiple biochemical pathways, including those involving adipokines, may mediate the effects of obesity. However, this could not be explored in this study due to cost constraints. Large population-based studies are needed to extrapolate the findings of the present study to the general population.

CONCLUSIONS

To conclude, we believe that using BMI-based WHO criteria for obesity classification may lead to an incorrect estimation of obesity in the population from the SEA region. In addition, this can have a bearing on the relationship between obesity and autonomic imbalance. Therefore, we propose that future research should explore autonomic imbalance due to obesity, keeping in mind the revised classification for Southeast Asian and Indian populations. Additionally, exploring other anthropometric-based indices, in conjunction with BMI (45), may provide a more comprehensive understanding of the magnitude of obesity in our subcontinent and its adverse effects on various organ systems, particularly the autonomic nervous system. ❑

Conflicts of interest: none declared.
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