

Title: Relationship between autonomic nervous system dysregulation, psychological distress, and sleep quality among university students

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Reviewer A: Dr Sifat E Syed , **ORCID:** 0000-0003-3075-0294 , **COI:** None, **AI disclosure:** None

- 1. Comment** The title mentions university students, but the manuscript suggests the students were from medical school (this is not clear in the methodology). Most of the cited literature is also for medical students. If that is the case, the title can be revised to mention 'medical' students instead of university students, as it is evidenced that medical students are more vulnerable to developing mental health problems.

Response: We thank the reviewer for this valuable suggestion. We agree that the study population specifically consisted of medical students, and the title has been revised accordingly for better precision and clarity.
Revised title:
"Relationship between autonomic nervous system dysregulation, psychological distress, and sleep quality among medical students: A cross-sectional mediation study" (Lines 2 - 3).
- 2. Comment** The Abstract is well written but completely AI-generated. The result section can include some numbers (CI, OR or r)

Response: The abstract has been revised to include important quantitative findings, including correlation coefficients, mediation percentage, beta coefficient, and 95% confidence interval in the Results section.
For example:
"accounting for 41% of the total effect ($\beta = -0.16$, 95% CI: -0.25 to -0.09 (Lines 48 - 52).
- 3. Comment** Objectives in Lines 109-110 can be simplified in language that will increase readability

Response: The objectives in the Introduction have been rewritten in simplified and clearer academic language. The objectives are now explicitly stated as:
Primary objective, Secondary objectives and Hypothesis (Lines 119 - 129).
- 4. Comment** Neurobiologically, ANS dysregulation is associated with sleep problems and psychological distress, as the HPA axis dysfunction is one of the core aetiologies, and this association is well established. The rationale and novelty statement need to be stronger.

Response: The Introduction has been strengthened by explicitly emphasizing the novelty of integrating:

 - objective HRV markers
 - subjective autonomic symptoms
 - psychological distress
 - mediation pathway analysis

within a medical student population (Lines 119 - 129).
- 5. Comment** The ethical concern is addressed, but the Methods section is too brief. It needs much more detail on the data collection procedure, population and sample, outcome measures, and data analysis.

Data collection procedure: Describe the stratified random sampling process (a flow chart may help), whether students were from medical school only or from both medical and technical universities, the year of study, and whether they lived in hostels or at home.

Outcome measures: State whether the instruments were validated or used the original English version, the number of items in each scale, the duration for applying each scale, and whether the measures were respondent- or interviewer-based. Specify the device used for ECG and how anonymity or confidentiality was maintained. Who collected the data, and was there any inter-rater variation?

Line 122: 'unwillingness to participate' is not an exclusion criterion

Line 136: Alcohol use was included in confounders. How about smoking and other substances?

Response: The Methods section has been substantially expanded.

Additional details now include:

 - stratified random sampling procedure
 - study population (MBBS students aged 18–25 years)
 - year-wise selection process
 - data collection protocol
 - acclimatization period
 - HRV device details
 - sampling rate

- recording posture
- software used
- confidentiality procedures
- trained investigator involvement
- total duration of assessment

We also revised the exclusion criteria by removing wording related to “unwillingness to participate” and clarified inclusion criteria accordingly (Lines 136 - 175).

6. Comment The study design is mentioned, but clarity and appropriateness are not described

Response: The study design has now been explicitly described as a: cross-sectional mediation study This is now clearly stated in both the title and Methods section (Title section, Lines 2 -3 and Methods sections, Lines131).

7. Comment No description of data analysis or statistical methods in the methods section

Response: A dedicated Statistical Analysis subsection has now been added, which includes mediation analysis approach (Lines 183 - 197).

8. Comment Use of uniform formatting for all tables is recommended

Response: All tables have been reformatted to ensure uniform structure, alignment, footnotes, and abbreviation definitions (Lines 483 - 502).

9. Comment The Figure 2 caption can be simplified as it's a simple bar chart. Figure 1 may not be necessary.

Response: References: Some are duplicated, many are irrelevant to rehabilitation systems, and they are formatting inconsistent.

Based on reviewer recommendations and to improve manuscript conciseness, figures have been removed from the revised version, and key findings are now adequately represented through tables and narrative text (Lines 483 – 502).

10. Comment There is significant redundancy between text and tables 2, 3 and 4

Response: The Results section has been carefully revised to reduce redundancy between text and tables.

The narrative now focuses on key findings and trends while detailed numerical values are presented in the tables (Lines 208 - 220).

Reviewer B: Anonymous, ORCID: 0009-0000-5671-4457, COI: None, AI disclosure: AI tools are used to assist in improving sentence and language clarity only.

11. Comment The title : "Relationship between autonomic nervous system dysregulation, psychological distress, and sleep quality among university students" — is largely appropriate and clearly communicates the three core constructs and the study population. However, it does not convey the study design (cross-sectional), nor the mechanistic (mediational) nature of the analysis, which is a key contribution. The authors may consider revising to something like: "Autonomic nervous system dysregulation, psychological distress, and sleep quality among university students: A cross-sectional mediation study."

Response: Thank you for this valuable suggestion. The title has been revised to reflect both the study design and mediation analysis. The revised title is "Relationship between autonomic nervous system dysregulation, psychological distress, and sleep quality among medical students: A cross-sectional mediation study".

12. Comment The abstract is well-structured using IMRAD format. The background concisely frames the problem, and the conclusions are supported by the results. However, the following issues require attention:

- The Methods section of the abstract states a pre-registered cross-sectional design but the main text does not include a trial registration number or registry name (e.g., ClinicalTrials.gov, OSF). This should be stated explicitly in both the abstract and methods.
- The Results section of the abstract reports that "psychological distress partially mediated" the ANS–sleep relationship but does not report the percentage mediated (41%) or confidence intervals, which are available in the main text. Including this key quantitative finding would strengthen the abstract.
- The abstract word count of 174 appears within the typical limit, but the keyword list uses non-standard capitalisation (e.g., "Pittsburgh Sleep Quality Index" should be capitalised consistently).

Response: The abstract has been revised to include:

- mediation percentage
- beta coefficient
- confidence interval
- clearer keyword formatting

Regarding pre-registration, we respectfully clarify that this was not a prospectively registered study, and the wording has been revised to remove any ambiguity (Lines 37 - 62).

- 13. Comment** The authors are encouraged to state a clear, testable primary objective (e.g., to examine the association between ANS dysregulation and sleep quality, and whether this relationship is mediated by psychological distress) and secondary objectives. The hypothesis derived from the neurovisceral integration model is mentioned, but could be more precisely worded to align with the mediation analysis approach.

Response: A clear primary objective, secondary objectives, and hypothesis have been formally stated in the Introduction (Lines 119-129).

- 14. Comment** The Introduction is generally well-written, engaging, and provides a solid theoretical framework anchored in the neurovisceral integration model. The flow from general context to specific gap is logical. There are inappropriate citation; ref. 5, 13 and 15.

The Introduction could more explicitly articulate the gap: few studies have integrated objective ANS biomarkers AND subjective autonomic symptoms with mediation analysis in students. This gap statement is partially made at the end of the section but could be sharpened.

Response: Thank you for highlighting these citation and gap issues.

References have been corrected (e.g., prevalence now cites Rotenstein et al. ; HRV-academic performance updated; DOI fixed); research gap sharpened to emphasize integration of objective ANS biomarkers, subjective symptoms, and mediation in students (Lines 76, 101, 107, 119-129).

- 15. Comment** Several important details are missing or unclear in the Methods section:

- HRV Recording:** The method states that 5-minute resting ECG was recorded using "validated wearable sensors." The specific device(s) used, sampling rate, recording posture (supine, sitting), time of day, and post-meal/exercise restrictions should be specified. Readers need sufficient detail to replicate the HRV measurement protocol.
- HRV Analysis:** RMSSD and LF/HF ratio are reported. Authors should state which HRV analysis software was used, whether data were visually inspected for artefacts, and whether frequency-domain analyses used FFT or autoregressive modelling.
- COMPASS-31:** The questionnaire is described as measuring autonomic symptoms across "six domains" but these domains are not listed. A brief description of what COMPASS-31 domains cover would improve clarity for readers unfamiliar with this tool.
- Mediation and SEM analyses:** The statistical approach is described briefly in the abstract but is almost entirely absent in the Methods section. The authors should clearly state: (i) software used (e.g., R, SPSS PROCESS macro, lavaan), (ii) bootstrapping parameters (5,000 resamples are noted in Table 5 but not in Methods), (iii) criteria for model fit indices reported in the SEM (CFI, TLI, RMSEA), and (iv) how variables were treated (continuous vs. categorical) in the mediation model.
- Physical activity** was measured using IPAQ-SF but the scoring method (categorical vs. MET-minutes) is not described. Similarly, "daily screen time" measurement is not specified (self-report, device-measured?).

Response: The Methods section has been revised to improve clarity, completeness, and reproducibility. Detailed information on HRV recording has been added, including device specification, sampling rate, posture, time of recording, and pre-assessment restrictions. HRV analysis procedures have been clarified by specifying the software used, artefact handling, and use of Fast Fourier Transform (FFT) for frequency-domain analysis.

The description of the COMPASS-31 questionnaire has been expanded to include its six domains. The statistical analysis section has been substantially revised to include details on mediation and structural equation modeling, including software, bootstrapping parameters (5,000 resamples), model fit criteria (CFI, TLI, RMSEA), and treatment of variables. Additionally, the scoring method for physical activity (IPAQ-SF; MET-minutes) and the assessment of daily screen time (self-reported) have been clarified (Lines 145–210).

- 16. Comment** The cross-sectional design is appropriate for the stated objectives of examining prevalence and associations. However, the cross-sectional design fundamentally limits causal or mediational inference — this is acknowledged in the Limitations but should also be tempered in the framing of the Results and Discussion, where causal language (e.g., "contributes to," "leads to," "disrupts") is used repeatedly.

Response: We acknowledged this limitation in the Limitations section, we have now revised the Results and Discussion sections to avoid causal language and ensure that all findings are presented as associations rather than causal effects. Terms such as "contributes to," "leads to," and "disrupts" have been replaced with more appropriate language (e.g., "is associated with," "is related to," "may be linked with"). Additionally, clarifying statements have been incorporated in the Results and Discussion to emphasize the cross-sectional nature of the study and the inability to infer directionality or causality (Lines 230–310).

- 17. Comment** The statistical approach is largely appropriate, combining correlation, multivariable regression, mediation, and SEM. However, several issues require clarification:

- The type of regression used is not stated. Given that PSQI and DASS-21 are ordinal/skewed scores, the assumption of normality should be tested and reported.
- In Table 3 (regression predicting DASS-21), the SE for "Female sex" is 0.89 — this is unusually large for a binary predictor (0/1) and may indicate an error or unstandardized coefficient. Authors should verify and clarify the units of SE values in regression tables.

- c) The LF/HF ratio was not included as a predictor in Table 4 (PSQI regression), despite being significant in Table 3. Was this a deliberate modelling decision? If so, the rationale should be stated.
- d) Variance inflation factors (VIF) or tolerance should be reported to rule out multicollinearity, given the inter-correlations between RMSSD, LF/HF, and COMPASS-31 (Table 2).
- e) The SEM fit indices (CFI = 0.96, TLI = 0.95, RMSEA = 0.041) are reported only in the Results section narrative. A formal SEM results table or figure path diagram with coefficients would considerably improve transparency.

Response: The manuscript has been revised to improve clarity and transparency. The type of regression (ordinary least squares) has now been explicitly stated, and assessment of normality (Shapiro–Wilk test and visual inspection) has been reported. The SE value for “Female sex” in Table 3 has been verified and corrected, and units for SE are now clearly specified in all regression tables.

The exclusion of LF/HF ratio from the PSQI regression model has been clarified as a deliberate decision due to collinearity, and this rationale is now stated in the Methods and table footnote. Multicollinearity diagnostics (variance inflation factor and tolerance values) have been added.

SEM reporting has been strengthened by including model fit criteria in the Methods and presenting results more transparently (Lines 180–240).

18. Comment Five tables are provided and collectively cover the key findings. The tables are generally clear and appropriately formatted. The following improvements are suggested:

- a) Table 1: Row labels are informative, but the table lacks a footnote defining abbreviations (RMSSD, LF/HF, COMPASS-31, DASS-21, PSQI). An empty last row appears to be a formatting artifact and should be removed.
- b) Table 2: The correlation matrix is incomplete — the upper triangle is empty (not populated with dashes or “—”) which is acceptable, but a note explaining this should be included. The table does not report the LF/HF–PSQI correlation value explicitly; it appears as 0.33 in the Results text but is not clearly legible in the table.
- c) Tables 3 and 4: Units for SE values are not stated. As noted above, the SE of 0.89 for “Female sex” in Table 3 appears anomalous and should be verified. The footnote “Model $R^2 = 0.42$ ” should be incorporated within the table rather than placed below as a standalone bold line.
- d) Table 5: This is well-structured and informative. The bootstrapping resampling number (5,000) noted here should also be stated in the Methods section.

Response: Table 1 has been revised to include a footnote defining all abbreviations, and the formatting artifact has been removed. Table 2 now includes a clarifying note regarding the lower triangle display and improved readability of correlation values. Tables 3 and 4 have been revised to include units for SE values, and the previously noted SE discrepancy has been corrected. Model fit statistics (R^2) have been incorporated within the tables. Table 5 remains unchanged, but the bootstrapping parameter (5,000 resamples) has now been explicitly stated in the Methods section (Lines 245–310).

19. Comment There is moderate redundancy between the Results narrative and the tables. For example, the correlation coefficients in the Results section (paragraph 2) largely repeat the values in Table 2. The authors may consider streamlining the Results narrative to highlight key findings and patterns, directing readers to tables for detailed values, rather than repeating each coefficient in the text. This would reduce manuscript length and improve readability.

Response: The Results section has been streamlined to reduce redundancy with tables. Detailed numerical values have been minimized in the text, with emphasis placed on key findings and interpretation, while directing readers to tables for full results (Lines 230–280).

20. Comment The Discussion is well-organised and logically structured, with subheadings for implications, limitations, and future directions. It appropriately situates findings within the neurovisceral integration model framework. However, the following points require attention:

- a) The authors use causal language extensively (e.g., “reduced vagal tone may impair...”, “autonomic dysregulation contributes to...”, “autonomic regulation leading to heightened emotional reactivity”) despite the cross-sectional design. This should be moderated throughout with language such as “is associated with” or “may be related to”.
- b) The comparison with prior studies is informative but could more directly engage with the specific limitations of those studies to contextualize why this study’s multi-method approach is a methodological advance.
- c) The 41% mediation by psychological distress is discussed, but the remaining 59% direct effect of RMSSD on PSQI is not substantively explained. The authors briefly mention “distress-independent pathways” but a more developed discussion of what these may be (nocturnal autonomic regulation, sleep architecture, circadian factors) would be valuable.

Response: We thank the reviewer for these insightful comments. The Discussion has been revised to avoid causal language and to consistently reflect the associational nature of the findings. Comparisons with prior studies have been strengthened by highlighting their methodological limitations and positioning the present study’s multi-method approach as an advancement.

The discussion of mediation has been expanded to address the unexplained direct effect (59%), with additional consideration of potential mechanisms such as nocturnal autonomic regulation, sleep architecture, and circadian influences (Lines 280–350).

21. Comment Strengths are implicitly acknowledged throughout the Discussion but are not formally summarised. The authors should consider a brief Strengths subsection. The Limitations section is appropriately self-critical and acknowledges the most important concerns (cross-sectional design, resting-only HRV, residual confounding). The authors should additionally acknowledge: (i) recruitment from a single institution limits generalizability, (ii) reliance on self-reported questionnaires for sleep and distress introduces reporting bias, and (iii) the acknowledgement of AI tool use (ChatGPT) for language editing — while commendable for transparency — raises questions about whether the use of AI-assisted writing complies with the target journal's policies, which should be verified.

Response: A dedicated Strengths subsection has now been added. The Limitations section has been expanded to include additional considerations, including single-institution recruitment, reliance on self-reported measures, and potential reporting bias. The statement regarding AI-assisted language editing has been reviewed to ensure compliance with journal policies (Lines 350–380).

22. Comment The conclusion is generally supported by the data; however, it slightly overstates the evidence. Stating "robust evidence" is strong language for a cross-sectional study with inherent causal limitations. The conclusion should be moderated to reflect the associational and partially mediational (not fully established causal) nature of the findings. The recommendation to target autonomic regulation for intervention, while scientifically grounded, should be presented as hypothesis-generating rather than practice-ready.

Response: We agree that the conclusion should be moderated. The language has been revised to avoid overstatement (e.g., "robust evidence") and to reflect the observational nature of the study. Implications for intervention are now presented as hypothesis-generating rather than definitive (Lines 380–395).

23. Comment The reference list needs correction. The following specific issues require attention:
References 5,13,15,16 and 17 are either incorrect inappropriately cited or duplicated.

Response: Thank you for identifying the citation concerns. The reference list has been thoroughly reviewed and corrected. This includes correction of the prevalence citation, DOI mismatch, removal of duplicate COMPASS-31 references, and verification of neurovisceral model and HRV-related citations (Lines 365–409).

24. Comment The overall narrative of the manuscript is logical and coherent. A few sentences are overly complex and would benefit from editing. The use of an engaging, slightly informal opening ("University life can feel exhilarating — and overwhelming") is refreshing but may be inconsistent with the formal academic tone required by some journals; authors should verify style guidelines. The key messages box is a useful addition.

Response: The manuscript has been edited to improve clarity and maintain a consistent academic tone. Informal phrasing has been revised where necessary to align with journal style guidelines, while preserving readability (Throughout manuscript).

25. Comment With a main text of 2,094 words, the manuscript is concise. However, the Methods section is comparatively thin relative to the analytical complexity (mediation + SEM). The authors should expand the Methods section to adequately describe the statistical analysis pipeline. Some Results text could be condensed by referring readers to tables, which would offset the added Methods content and keep the overall length appropriate.

Response: The Methods section has been expanded to better reflect the analytical complexity, particularly for mediation and SEM analyses. To maintain overall length, the Results section has been condensed by reducing redundancy and referring readers to tables (Lines 145–280).

26. Comment The English is generally of publishable standard. The manuscript appears to have been professionally language-edited. Minor issues include Inconsistent use of quotation marks vs. italics for instrument names, missing possessive apostrophe, run-on sentences in the Discussion.

The authors acknowledge use of ChatGPT for language editing. While transparency is appreciated, the manuscript should be carefully reviewed by the corresponding author to ensure accuracy and that AI-generated phrasing does not inadvertently misrepresent the methods or findings.

Response: A Minor language issues have been corrected, including consistent formatting of instrument names, grammatical corrections (e.g., possessive forms), and improved sentence structure for clarity. The manuscript has been carefully reviewed to ensure accuracy and appropriateness of all phrasing. The use of AI has been disclosed in appropriate section.

Responsible editor: M Mostafa Zaman, **ORCID:** 0000-0002-1736-1342

27. Comment The introduction Section is very lengthy, Please reduce it to 50%

Response: Length has been reduced keeping the main points.

28. Comment Figure 1 is not required.

Response: Based on recommendations and to improve manuscript conciseness, figures have been removed from the revised version (Lines 483 – 502).

29. Comment More than half of the references are outdated (>10 years). Kindly use recent references, if available.

Response: We have carefully reviewed the reference list and replaced the majority of older citations with more recent studies published within the last ten years.