International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

Assessment Of Heart Rate Variability in First-Year MBBS Students During Academic Stress

Dr. Md Faisal Sultan[1], Dr. Shashi Bhushan[2], Dr. Arup Mondal[3], Dr. Maloy B. Mandal[4], Dr. B. M. Tengankai[5], Dr. Sirsha Majumder[6]

[1]Junior Resident, Department of Physiology, MGM Medical College & LSK Hospital, Kishanganj, Bihar, India.
[2]Junior Resident, Department of Physiology, MGM Medical College & LSK Hospital, Kishanganj, Bihar, India.
[3]Assistant Professor, Department of Physiology, MGM Medical College & LSK Hospital, Kishanganj, Bihar, India.
[4]Professor & H.O.D, Department of Physiology, MGM Medical College & LSK Hospital, Kishanganj, Bihar, India.
[5]Associate Professor, Department of Physiology, MGM Medical College & LSK Hospital, Kishanganj, Bihar, India.
[6]Assistant Professor, Department of Physiology, MGM Medical College & LSK Hospital, Kishanganj, Bihar, India.

OPEN ACCESS

Corresponding Author:

Dr. Md Faisal Sultan
Junior Resident, Department of Physiology, MGM Medical College & LSK Hospital, Kishanganj, Bihar – 855107, India.
E-mail:
DRFAISALSULTAN@gmail.com

Received: 22-05-2026
Accepted: 12-06-2026
Available online: 30-06-2026

ABSTRACT

Background: The transition into the first year of the MBBS course exposes students to a sustained and intense academic load, compressed assessment schedules and relocation away from family support. Such chronic psychosocial stress is known to perturb cardiac autonomic regulation. Heart rate variability (HRV), the beat-to-beat oscillation in successive R-R intervals, offers a simple, non-invasive and reproducible window onto sympathovagal balance and is increasingly used as an objective correlate of subjectively perceived stress.

Aim and Objectives: To assess short-term HRV in first-year MBBS students during a routine (non-examination) academic period and again immediately before a major university examination; to compare time-domain, frequency-domain and non-linear HRV indices between the two states; to examine gender-related differences in the autonomic response; and to correlate HRV indices with self-reported stress measured by the Perceived Stress Scale (PSS-10).

Materials and Methods: This prospective observational study was conducted in the Department of Physiology, MGM Medical College & LSK Hospital, Kishanganj, Bihar, over one year (January 2025 to December 2025). One hundred healthy first-year MBBS students (72 boys, 28 girls) aged 18–22 years were enrolled after written informed consent and institutional ethics committee approval. Each participant served as his or her own control. Perceived stress was quantified using the PSS-10. Short-term (5-minute) lead II electrocardiographic recordings were obtained in the supine position under standardised laboratory conditions between 09:00 and 11:00 h, first during a stress-free academic period and again within 48–72 hours preceding the examination. R-R interval series were analysed in accordance with the Task Force guidelines of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Data were analysed using paired t-test or Wilcoxon signed-rank test, unpaired t-test for gender comparison, and Pearson correlation; $p < 0.05$ was considered statistically significant.

Results: The mean age was 19.58 ± 1.00 years. PSS-10 scores rose from 14.18 ± 3.63 to 23.80 ± 4.87 ($p < 0.001$), and 33% of students entered the high perceived-stress band. Basal heart rate rose from 72.72 ± 4.99 to 81.26 ± 6.61 beats/min. SDNN fell from 48.59 ± 7.96 to 37.15 ± 9.00 ms, RMSSD from 42.58 ± 7.64 to 28.94 ± 9.64 ms and pNN50 from 20.86 ± 5.45 to 10.88 ± 6.12 % (all $p < 0.001$). LF power rose from 47.00 ± 4.73 to 62.82 ± 5.39 nu and HF power fell from 53.00 ± 4.73 to 37.18 ± 5.39 nu, with a rise in the LF/HF ratio from 0.90 ± 0.17 to 1.75 ± 0.47 ($p < 0.001$). Poincaré SD1 fell from 30.11 ± 5.40 to 20.46 ± 6.81 ms and SD2 from 61.58 ± 10.95 to 48.03 ± 12.23 ms (both $p < 0.001$). PSS-10 correlated

negatively with RMSSD (r = -0.541) and HF nu (r = -0.684) and positively with the LF/HF ratio (r = +0.624; all p < 0.001). Girls retained a higher SDNN (p = 0.036) despite a higher heart rate (p = 0.042), but perceived stress, normalised powers and the LF/HF ratio did not differ between the sexes.

Conclusion: Academic examination stress in first-year MBBS students was accompanied by a measurable shift in cardiac autonomic balance towards sympathetic predominance with parasympathetic withdrawal, reflected in reduced overall and vagally mediated HRV. Given the recognised association between depressed HRV and long-term cardiovascular risk, routine screening for academic stress and the incorporation of structured stress-reduction measures into the undergraduate medical curriculum merit serious consideration.

Keywords*: Academic stress; Autonomic nervous system; Heart rate variability; Medical students; Perceived Stress Scale; Sympathovagal balance.*

INTRODUCTION

Stress is the non-specific response of the body to any demand placed upon it, and it becomes pathologically relevant when the demand persists beyond the individual's capacity to adapt. Undergraduate medical education is widely acknowledged to be among the most demanding of professional courses. The first year of the MBBS programme is particularly taxing: students confront an unfamiliar volume of factual material, a dissection hall and a laboratory for the first time, frequent internal assessments culminating in a high-stakes university examination, and, for the great majority, separation from home and family support networks. Systematic reviews have repeatedly documented rates of psychological distress among medical students that exceed those of age-matched peers in the general population.[1]

The physiological consequences of such sustained psychosocial stress are mediated largely through two interconnected effector limbs: the hypothalamo-pituitary-adrenal axis and the sympatho-adrenomedullary system. Activation of the latter produces an increase in sympathetic outflow and a reciprocal withdrawal of cardiac vagal tone. While the acute response is adaptive, its repeated or prolonged elicitation is implicated in hypertension, endothelial dysfunction, arrhythmogenesis and accelerated atherosclerosis.[2] Identifying this autonomic shift at an early, still-reversible stage in a young and otherwise healthy population is therefore of considerable preventive importance.

Heart rate variability (HRV) is the physiological oscillation in the time interval between consecutive sinus beats. It arises predominantly from the continuous modulation of the sinoatrial node by the two divisions of the autonomic nervous system, and its analysis provides a quantitative, non-invasive and reproducible index of cardiac autonomic function.[3–5] Conventional analysis proceeds along three complementary lines. Time-domain measures such as the standard deviation of normal-to-normal intervals (SDNN), the root mean square of successive differences (RMSSD) and the proportion of successive intervals differing by more than 50 ms (pNN50) describe the magnitude of variability, the latter two being preferential markers of vagal activity. Frequency-domain analysis decomposes the tachogram into a low-frequency (LF, 0.04–0.15 Hz) component reflecting mixed sympathetic and parasympathetic influences modulated by the baroreflex and a high-frequency (HF, 0.15–0.40 Hz) component that is essentially vagal and respiration-linked; the ratio of the two is widely, if not uncontroversially, interpreted as an index of sympathovagal balance.[3] Non-linear methods such as the Poincaré plot characterise the complexity and self-similarity of the R-R series and are comparatively robust to non-stationarity in short recordings.[4]

A number of investigators have reported a reduction in overall and vagally mediated HRV during examination periods, in occupational stress and in experimentally induced mental arithmetic tasks.[6–9] However, the published Indian literature on first-year medical undergraduates remains limited, is often cross-sectional in design, and seldom examines gender-related differences or the concordance between objective autonomic measures and validated subjective stress instruments in the same cohort. Data from medical colleges in eastern India, and specifically from students drawn largely from the Seemanchal region of Bihar, are almost entirely lacking.

The present study was therefore undertaken to assess and compare short-term HRV indices in first-year MBBS students during a routine academic period and immediately preceding a major university examination, to examine whether the autonomic response differs between male and female students, and to determine whether objectively measured HRV correlates with subjectively perceived stress as assessed by the Perceived Stress Scale.

AIM AND OBJECTIVES

Aim

To assess cardiac autonomic function by means of short-term heart rate variability analysis in first-year MBBS students during academic stress.

Objectives

- To record and compare time-domain HRV indices (mean RR, mean HR, SDNN, RMSSD, NN50, pNN50) during a non-examination period and during the pre-examination period.
- To record and compare frequency-domain HRV indices (total power, VLF, LF, HF, LF nu, HF nu, LF/HF ratio) between the two states.
- To evaluate non-linear HRV indices derived from the Poincaré plot (SD1, SD2, SD1/SD2).
- To compare the autonomic response to examination stress between male and female students.
- To correlate HRV indices with the Perceived Stress Scale (PSS-10) score.

MATERIALS AND METHODS

Study design and setting

This was a prospective, observational, self-controlled (paired) study carried out in the Autonomic Function Testing Laboratory of the Department of Physiology, MGM Medical College & Lions Seva Kendra Hospital, Kishanganj, Bihar, India. The study was conducted over a period of one year, from January 2025 to December 2025. The manuscript has been prepared in accordance with the STROBE statement for observational studies.

Study population and sample size

One hundred apparently healthy first-year MBBS students of the institution, comprising 72 boys and 28 girls, were enrolled by complete enumeration of the eligible batch after screening against the criteria listed below. The minimum required sample size was calculated for the primary outcome variable, RMSSD, using the formula $n = (Z\alpha/2 + Z\beta)^2 \times SD^2 / d^2$ for paired observations. Assuming a minimum difference of interest (d) of 5 ms in RMSSD between the two states, a conservative standard deviation of the paired differences (SD) of 10 ms taken from previously published work on examination stress in medical undergraduates [Authors: cite the exact reference study from which this standard deviation was taken], a two-sided alpha of 0.05 and a power of 90%, the minimum required sample was 43 students. The whole batch of 100 consenting eligible students was studied, which comfortably exceeds this requirement and also permits the planned subgroup comparison between male and female participants.

Inclusion criteria

- First-year MBBS students of either sex, aged 18–22 years.
- Apparently healthy, with a normal general and systemic examination.
- Body mass index between 18.5 and 24.9 kg/m².
- Willing to give written informed consent and to attend both recording sessions.

Exclusion criteria

- Known cardiovascular, respiratory, endocrine, neurological, renal or hepatic disease; diabetes mellitus; hypertension.
- Any acute febrile or infective illness within two weeks preceding a recording.
- Regular use of any medication known to influence autonomic function, including beta-blockers, antihistaminics, antidepressants, anxiolytics, bronchodilators and corticosteroids.
- Diagnosed psychiatric illness or current psychotropic therapy.
- Smoking, tobacco chewing, alcohol consumption or substance use.
- Athletes and students engaged in structured endurance or resistance training, or in regular yoga, pranayama or meditation practice.
- Female students during the menstrual phase or with irregular menstrual cycles; recordings in female participants were scheduled during the mid-follicular phase (day 7–11) of the cycle wherever practicable.
- Electrocardiographic evidence of any rhythm other than sinus, or a recording containing more than 5% ectopic or artefactual beats.

Ethical considerations

The purpose and procedure of the study were explained to each participant in a language he or she understood, and written informed consent was obtained. Participation was entirely voluntary, refusal carried no academic consequence, and participants were free to withdraw at any stage. All data were anonymised by assigning a code number, and confidentiality was maintained throughout. The study conformed to the ethical principles of the Declaration of Helsinki and to the National Ethical Guidelines for Biomedical and Health Research involving Human Participants (ICMR, 2017).

Study protocol

Each participant was studied on two occasions and served as his or her own control:

- Session 1 (non-examination / baseline period): recorded during a routine teaching block with no scheduled internal assessment or university examination within the preceding or following four weeks.
- Session 2 (examination / stress period): recorded within 48 to 72 hours preceding the first paper of the university examination.

Participants were familiarised with the laboratory and the recording procedure on a separate preliminary visit in order to minimise anxiety attributable to the novelty of the environment itself.

Pre-recording instructions
All participants were instructed to abstain from tea, coffee, cola and any other caffeinated beverage for at least 12 hours, to avoid strenuous physical activity for 24 hours preceding the test, to have a light breakfast at least two hours before reporting, to empty the bladder before the recording and to obtain adequate sleep on the preceding night. Compliance was confirmed by direct enquiry before each session.

Assessment of perceived stress
Subjective stress was quantified at both sessions using the 10-item Perceived Stress Scale (PSS-10) of Cohen et al.[10], a validated and widely used instrument that measures the degree to which situations in one's life over the preceding month are appraised as unpredictable, uncontrollable and overloading. Each item is scored on a five-point Likert scale from 0 (never) to 4 (very often), with items 4, 5, 7 and 8 reverse-scored; the total score ranges from 0 to 40, higher scores indicating greater perceived stress. Scores of 0–13, 14–26 and 27–40 were interpreted as low, moderate and high perceived stress respectively.

Anthropometric and cardiovascular measurements
Height was measured to the nearest 0.5 cm with a stadiometer and weight to the nearest 0.1 kg on a calibrated digital weighing scale, with the participant barefoot and in light clothing; body mass index was calculated as weight in kilograms divided by the square of height in metres. Basal heart rate and blood pressure were recorded after 10 minutes of supine rest using a validated device, blood pressure being taken in the right arm at heart level; the mean of two readings taken five minutes apart was used. Pulse pressure, mean arterial pressure and rate-pressure product were derived by standard formulae.

Recording of heart rate variability
All recordings were made in a quiet, dimly lit, air-conditioned laboratory maintained at 24–26 °C, between 09:00 and 11:00 h, in order to eliminate the confounding influence of circadian variation in autonomic tone. After a 10-minute period of supine rest, a lead II electrocardiogram was acquired continuously for 5 minutes using [Authors: insert the exact make and model of the data acquisition system used, for example RMS Polyrite-D, AD Instruments PowerLab or BIOPAC MP series] at a sampling frequency of 1000 Hz. Participants were asked to lie still, to remain awake with the eyes open, to refrain from speaking and to breathe quietly and spontaneously at their own rate; respiratory rate was monitored and recordings in which it fell outside 12–20 breaths per minute were repeated.

The digitised R-R interval series was exported and analysed offline using Kubios HRV software[11] [Authors: insert version number]. Automatic beat detection was verified visually by a single observer; ectopic and artefactual beats were identified and corrected by piecewise cubic spline interpolation, and any recording requiring correction of more than 5% of beats was discarded and repeated. Very-low-frequency trend components were removed using the smoothness priors detrending method. Frequency-domain analysis was performed on the interpolated and evenly resampled tachogram by the fast Fourier transform (Welch periodogram) method. All definitions, recording conditions and interpretations conformed to the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.[3]

Heart rate variability parameters analysed
Time domain:
Mean RR interval (ms), mean heart rate (beats/min), SDNN (ms), RMSSD (ms), NN50 (count) and pNN50 (%).

Frequency domain:
Total power (ms²), very-low-frequency power (ms²), low-frequency power (ms² and normalised units), high-frequency power (ms² and normalised units) and the LF/HF ratio.

Non-linear:
Poincaré plot descriptors SD1 (ms), SD2 (ms) and the SD1/SD2 ratio.

Statistical analysis
Data were compiled in Microsoft Excel and analysed using SPSS version [Authors: insert the version actually used]. The normality of distribution of each variable was tested by the Shapiro–Wilk test. Normally distributed data are expressed as mean ± standard deviation and skewed data as median with interquartile range; categorical data are expressed as frequency and percentage. Comparison between the non-examination and examination sessions was made using the paired t-test for normally distributed variables and the Wilcoxon signed-rank test for skewed variables. Comparison between male and female students was made using the unpaired (independent-samples) t-test with Welch's correction or the Mann–Whitney U test as appropriate. Frequency-domain powers, which are characteristically skewed, were subjected to natural logarithmic transformation before parametric analysis. The magnitude of the within-subject change was additionally expressed as the standardised effect size for paired data (Cohen's d_z). The relationship between PSS-10 scores and HRV indices was

examined by Pearson's correlation coefficient (or Spearman's rank correlation for non-normally distributed data). A two-tailed p value of less than 0.05 was considered statistically significant.

RESULTS

A total of 100 first-year MBBS students (72 boys, 72.0% and 28 girls, 28.0%) completed both recording sessions; there were no dropouts and no recording was discarded for excessive ectopy or artefact. The mean age of the cohort was 19.58 ± 1.00 years and the mean body mass index was 21.65 ± 1.46 kg/m². The baseline demographic and anthropometric characteristics of the study population are presented in Table 1. Boys and girls were closely comparable in age (19.58 ± 1.03 vs 19.57 ± 0.92 years, p = 0.829) and in body mass index (21.73 ± 1.51 vs 21.46 ± 1.35 kg/m², p = 0.384). As expected, boys were significantly taller (169.31 ± 5.03 vs 159.38 ± 5.33 cm), heavier (62.38 ± 5.98 vs 54.59 ± 5.27 kg) and had a larger body surface area (1.71 ± 0.10 vs 1.55 ± 0.10 m²; all p < 0.001). Since each participant served as his or her own control, these anthropometric differences do not confound the within-subject comparisons that follow.

Table 1: Baseline demographic and anthropometric characteristics of the study population (n = 100)

Variable	Boys (n = 72)	Girls (n = 28)	Total (n = 100)	p value
Age (years)	19.58 ± 1.03	19.57 ± 0.92	19.58 ± 1.00	0.829
Height (cm)	169.31 ± 5.03	159.38 ± 5.33	166.53 ± 6.78	< 0.001*
Weight (kg)	62.38 ± 5.98	54.59 ± 5.27	60.20 ± 6.75	< 0.001*
Body mass index (kg/m²)	21.73 ± 1.51	21.46 ± 1.35	21.65 ± 1.46	0.384
Body surface area (m²)	1.71 ± 0.10	1.55 ± 0.10	1.67 ± 0.12	< 0.001*

Values are expressed as mean ± standard deviation. p value derived from the unpaired t-test; p < 0.05 considered significant.

Perceived stress rose markedly during the examination period. The mean PSS-10 score increased from 14.18 ± 3.63 at baseline to 23.80 ± 4.87 before the examination (p < 0.001), a relative rise of 67.8% and a very large effect (Cohen's d_2 = 4.32). At baseline 40 students (40.0%) fell in the low and 60 (60.0%) in the moderate perceived-stress band, and none in the high band; before the examination no student remained in the low band, 67 (67.0%) were in the moderate band and 33 (33.0%) had crossed into the high perceived-stress category. This was accompanied by a significant rise in basal heart rate (72.72 ± 4.99 to 81.26 ± 6.61 beats/min), systolic blood pressure (114.62 ± 5.95 to 121.72 ± 6.96 mmHg), diastolic blood pressure (72.25 ± 4.75 to 77.29 ± 5.59 mmHg) and rate-pressure product (83.36 ± 7.19 to 98.96 ± 10.26 ×10²), as shown in Table 2. Mean respiratory rate rose slightly but remained within the pre-specified 12–20 breaths/min window in every recording (15.17 ± 1.03 to 16.78 ± 1.12 breaths/min).

Table 2: Perceived stress and basal cardiovascular parameters during the non-examination and examination periods (n = 100)

Parameter	Non-examination period	Examination period	% change	p value
PSS-10 score	14.18 ± 3.63	23.80 ± 4.87	+67.8	< 0.001*
Heart rate (beats/min)	72.72 ± 4.99	81.26 ± 6.61	+11.8	< 0.001*
Systolic blood pressure (mmHg)	114.62 ± 5.95	121.72 ± 6.96	+6.2	< 0.001*
Diastolic blood pressure (mmHg)	72.25 ± 4.75	77.29 ± 5.59	+7.0	< 0.001*
Mean arterial pressure (mmHg)	86.37 ± 3.94	92.10 ± 4.70	+6.6	< 0.001*
Pulse pressure (mmHg)	42.37 ± 7.14	44.43 ± 8.17	+4.8	< 0.001*
Rate-pressure product (×10²)	83.36 ± 7.19	98.96 ± 10.26	+18.7	< 0.001*
Respiratory rate (breaths/min)	15.17 ± 1.03	16.78 ± 1.12	+10.6	< 0.001*

Values are expressed as mean ± standard deviation. p value derived from the paired t-test / Wilcoxon signed-rank test.

All time-domain indices of heart rate variability declined significantly during the examination period (Table 3); no time-domain parameter failed to reach statistical significance. The reduction was most pronounced in the vagally mediated indices, pNN50 falling by 47.9% (20.86 ± 5.45 to 10.88 ± 6.12 %) and RMSSD by 32.0% (42.58 ± 7.64 to 28.94 ± 9.64 ms), indicating withdrawal of parasympathetic modulation of the sinoatrial node. The smallest proportional change among the variability measures was in SDNN, which fell by 23.5% (48.59 ± 7.96 to 37.15 ± 9.00 ms); the mean RR interval shortened by 11.4% (833.60 ± 59.10 to 738.16 ± 61.02 ms) with a corresponding 13.1% rise in mean heart rate. The effect sizes for the fall in RMSSD and SDNN were both large (Cohen's d_2 = -3.21).

Table 3: Comparison of time-domain heart rate variability indices between the non-examination and examination periods (n = 100)

Parameter	Non-examination period	Examination period	% change	p value
Mean RR interval (ms)	833.60 ± 59.10	738.16 ± 61.02	-11.4	< 0.001*
Mean heart rate (beats/min)	72.33 ± 5.08	81.83 ± 6.73	+13.1	< 0.001*
SDNN (ms)	48.59 ± 7.96	37.15 ± 9.00	-23.5	< 0.001*

RMSSD (ms)	42.58 ± 7.64	28.94 ± 9.64	-32.0	< 0.001*
NN50 (count)	74.80 ± 19.10	43.50 ± 23.89	-41.8	< 0.001*
pNN50 (%)	20.86 ± 5.45	10.88 ± 6.12	-47.9	< 0.001*

SDNN: standard deviation of normal-to-normal RR intervals; RMSSD: root mean square of successive RR interval differences; NN50: number of successive RR intervals differing by more than 50 ms; pNN50: NN50 expressed as a percentage of total RR intervals. Values are mean ± SD; paired t-test / Wilcoxon signed-rank test.

Frequency-domain analysis demonstrated a reciprocal redistribution of spectral power (Table 4). Total power and absolute high-frequency power fell, low-frequency power in normalised units rose and high-frequency power in normalised units fell, with a consequent significant increase in the LF/HF ratio. Taken together these changes indicate a shift of sympathovagal balance towards sympathetic predominance during examination stress.

Table 4: Comparison of frequency-domain heart rate variability indices between the non-examination and examination periods (n = 100)

Parameter	Non-examination period	Examination period	% change	p value
Total power (ms²)	2892.75 ± 746.15	2038.77 ± 597.92	-29.5	< 0.001*
VLF power (ms²)	575.06 ± 174.04	477.41 ± 162.75	-17.0	< 0.001*
LF power (ms²)	1092.90 ± 360.87	983.58 ± 374.81	-10.0	< 0.001*
HF power (ms²)	1224.80 ± 388.75	577.78 ± 220.47	-52.8	< 0.001*
LF power (nu)	47.00 ± 4.73	62.82 ± 5.39	+33.7	< 0.001*
HF power (nu)	53.00 ± 4.73	37.18 ± 5.39	-29.9	< 0.001*
LF/HF ratio	0.90 ± 0.17	1.75 ± 0.47	+94.6	< 0.001*

VLF: very low frequency (≤0.04 Hz); LF: low frequency (0.04–0.15 Hz); HF: high frequency (0.15–0.40 Hz); nu: normalised units. Absolute powers were log-transformed before analysis. Values are mean ± SD; paired t-test / Wilcoxon signed-rank test.

Non-linear analysis of the Poincaré plot (Table 5) showed a reduction in SD1, the index of short-term beat-to-beat variability that closely parallels RMSSD and reflects vagal activity, from 30.11 ± 5.40 ms to 20.46 ± 6.81 ms (-32.0%, $p < 0.001$), together with a smaller but still significant fall in SD2, the measure of long-term variability, from 61.58 ± 10.95 ms to 48.03 ± 12.23 ms (-22.0%, $p < 0.001$). Because the short-axis descriptor contracted proportionately more than the long-axis descriptor, the SD1/SD2 ratio also fell significantly, from 0.50 ± 0.09 to 0.44 ± 0.14 (-12.2%, $p < 0.001$), so that the Poincaré cloud became narrower and more elongated — the characteristic geometric signature of vagal withdrawal with relative preservation of slower, sympathetically and baroreflex-mediated oscillations.

Table 5: Comparison of non-linear (Poincaré plot) heart rate variability indices between the non-examination and examination periods (n = 100)

Parameter	Non-examination period	Examination period	% change	p value
SD1 (ms)	30.11 ± 5.40	20.46 ± 6.81	-32.0	< 0.001*
SD2 (ms)	61.58 ± 10.95	48.03 ± 12.23	-22.0	< 0.001*
SD1/SD2 ratio	0.50 ± 0.09	0.44 ± 0.14	-12.2	< 0.001*

SD1: standard deviation of the Poincaré plot perpendicular to the line of identity (short-term variability); SD2: standard deviation along the line of identity (long-term variability). Values are mean ± SD; paired t-test / Wilcoxon signed-rank test.

Comparison between male and female students during the examination period is presented in Table 6. Perceived stress at the time of the examination was virtually identical in the two groups (23.69 ± 5.24 in boys vs 24.07 ± 3.82 in girls, $p = 0.693$). Girls maintained a significantly higher overall variability, SDNN being 39.88 ± 7.29 ms against 36.08 ± 9.41 ms in boys ($p = 0.036$), despite a significantly faster mean heart rate (83.90 ± 5.90 vs 81.03 ± 6.90 beats/min, $p = 0.042$). The vagal indices were numerically higher in girls but did not reach statistical significance (RMSSD 31.52 ± 9.98 vs 27.93 ± 9.38 ms, $p = 0.108$; pNN50 12.78 ± 6.08 vs 10.14 ± 6.02 %, $p = 0.056$; SD1 22.29 ± 7.06 vs 19.75 ± 6.63 ms, $p = 0.108$), and the normalised spectral powers and the LF/HF ratio did not differ between the sexes (LF/HF 1.70 ± 0.44 in girls vs 1.78 ± 0.48 in boys, $p = 0.343$). When the magnitude of change from baseline was compared instead of the absolute examination-period values, boys and girls showed an equivalent rise in PSS-10 score (9.49 ± 2.23 vs 9.96 ± 2.24, $p = 0.341$) and equivalent falls in RMSSD ($p = 0.124$) and HF nu ($p = 0.554$) and rise in LF/HF ratio ($p = 0.838$); only the fall in SDNN was significantly greater in boys (-12.05 ± 3.72 vs -9.87 ± 2.59 ms, $p = 0.001$). The autonomic response to the stressor was therefore qualitatively the same in both sexes and differed only marginally in magnitude.

Table 6: Gender comparison of heart rate variability indices during the examination period

Parameter	Boys (n = 72)	Girls (n = 28)	p value
PSS-10 score	23.69 ± 5.24	24.07 ± 3.82	0.693
Mean heart rate (beats/min)	81.03 ± 6.90	83.90 ± 5.90	0.042*
SDNN (ms)	36.08 ± 9.41	39.88 ± 7.29	0.036*
RMSSD (ms)	27.93 ± 9.38	31.52 ± 9.98	0.108
pNN50 (%)	10.14 ± 6.02	12.78 ± 6.08	0.056
LF power (nu)	63.03 ± 5.70	62.27 ± 4.54	0.343
HF power (nu)	36.97 ± 5.70	37.73 ± 4.54	0.343
LF/HF ratio	1.78 ± 0.48	1.70 ± 0.44	0.343
SD1 (ms)	19.75 ± 6.63	22.29 ± 7.06	0.108

Values are mean ± SD; unpaired t-test / Mann–Whitney U test.

Correlation analysis (Table 7) revealed that the PSS-10 score during the examination period was inversely related to the vagally mediated indices RMSSD (r = -0.541), pNN50 (r = -0.590), HF nu (r = -0.684) and SD1 (r = -0.541), and to overall variability as measured by SDNN (r = -0.480), and directly related to the LF/HF ratio (r = +0.624), to LF nu (r = +0.684) and to mean heart rate (r = +0.590); all correlations were significant at $p < 0.001$. The same relationship was present, though weaker, at baseline (PSS-10 vs RMSSD, r = -0.429, $p < 0.001$). This concordance between the subjective appraisal of stress and objectively measured cardiac autonomic modulation lends internal validity to the findings. It should be noted that SD1 is mathematically a scaled equivalent of RMSSD (SD1 = RMSSD/√2), so their correlation coefficients with PSS-10 are necessarily identical and the two indices do not constitute independent confirmation of one another.

Table 7: Correlation of PSS-10 score with heart rate variability indices during the examination period (n = 100)

HRV index	Correlation coefficient (r)	p value	Interpretation
Mean heart rate	+0.590	< 0.001*	Moderate positive correlation
SDNN	-0.480	< 0.001*	Moderate negative correlation
RMSSD	-0.541	< 0.001*	Moderate negative correlation
pNN50	-0.590	< 0.001*	Moderate negative correlation
LF (nu)	+0.684	< 0.001*	Strong positive correlation
HF (nu)	-0.684	< 0.001*	Strong negative correlation
LF/HF ratio	+0.624	< 0.001*	Strong positive correlation
SD1	-0.541	< 0.001*	Moderate negative correlation

Pearson correlation coefficient (Spearman where distribution was non-normal); $p < 0.05$ considered significant.
The principal findings are illustrated in Figure 1 and Figure 2.

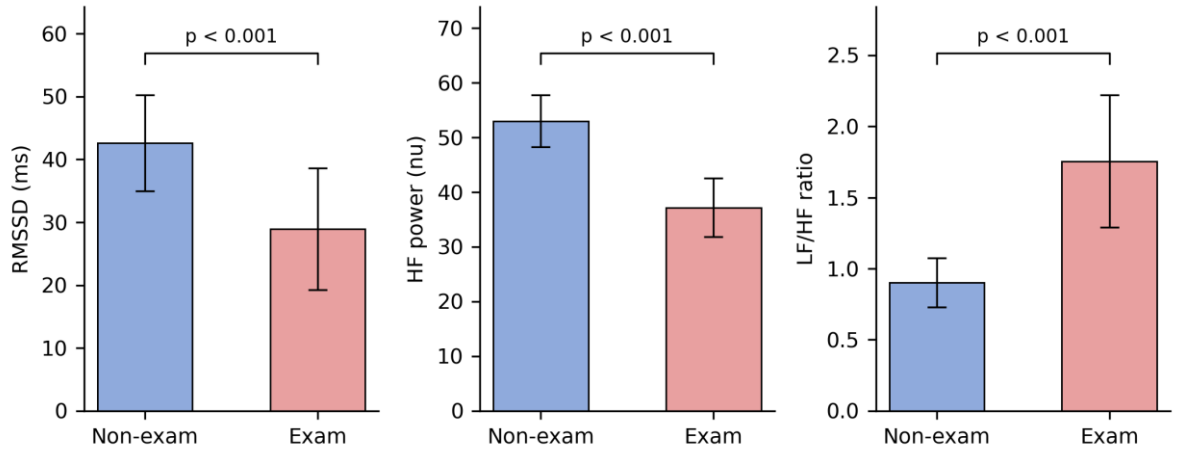

Figure 1: Comparison of RMSSD, high-frequency power in normalised units and the LF/HF ratio between the non-examination and examination periods (n = 100). Bars represent mean values and error bars one standard deviation. All three comparisons were significant on the paired t-test or Wilcoxon signed-rank test ($p < 0.001$).

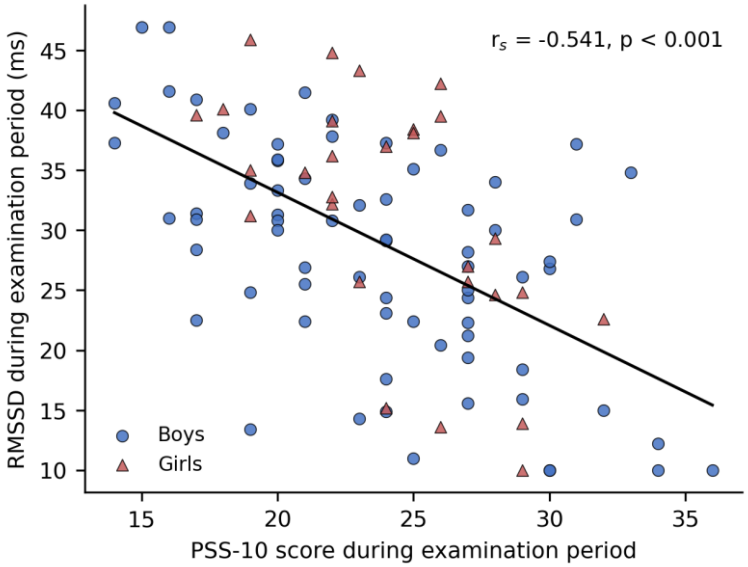

Figure 2: Scatter plot of the Perceived Stress Scale (PSS-10) score against RMSSD during the examination period (n = 100), with the fitted least-squares regression line. Boys are shown as circles and girls as triangles. Spearman's rank correlation coefficient $r_s = -0.541$, $p < 0.001$.

DISCUSSION

The principal finding of the present study is that the approach of a major university examination was accompanied in first-year MBBS students by a consistent and statistically significant depression of heart rate variability, characterised by a fall in overall variability, a disproportionate fall in the vagally mediated indices, and a relative increase in low-frequency spectral power. This constellation of changes is the electrocardiographic signature of sympathetic activation with concomitant parasympathetic withdrawal.

The reduction observed in RMSSD, pNN50, HF power in normalised units and Poincaré SD1 is of particular physiological interest, because these four indices share a common dependence on respiratory sinus arrhythmia and are the most specific non-invasive markers of efferent cardiac vagal traffic.[4] Their concurrent decline is difficult to attribute to anything other than a genuine reduction in tonic vagal restraint on the sinoatrial node. The accompanying rise in the LF/HF ratio is consistent with a shift in sympathovagal balance, although this ratio must be interpreted with caution: low-frequency power is not a pure index of sympathetic outflow, being influenced by baroreflex gain, respiratory pattern and vagal activity as well.[3,4]

The mechanism underlying these observations is well characterised. Appraisal of the examination as threatening activates limbic structures, principally the amygdala and the medial prefrontal cortex, which project to the hypothalamic paraventricular nucleus and thence to brainstem cardiovascular nuclei. The resulting increase in sympathetic outflow from the rostral ventrolateral medulla and the inhibition of the nucleus ambiguus produce tachycardia, increased myocardial contractility and peripheral vasoconstriction. Simultaneous activation of the hypothalamo-pituitary-adrenal axis elevates circulating cortisol, which sensitises the cardiovascular system to catecholamines and further attenuates vagal tone. According to the neurovisceral integration model, prefrontal inhibitory control over these subcortical structures is itself impaired under conditions of perceived threat and cognitive load, leading to disinhibition of sympathoexcitatory circuits and a fall in HRV.[2,12]

Our findings are in broad agreement with the published literature. Reductions in short-term HRV during examination periods have been reported in medical and other university students in several settings, and meta-analytic evidence confirms that both acute and chronic psychological stress are associated with reduced HRV, the association being strongest for vagally mediated indices.[6,7,9] Studies of real-life occupational and academic stressors have similarly documented sympathetic predominance with a rise in the LF/HF ratio and a fall in high-frequency power.[8] The direction of every change observed in the present cohort — a fall in SDNN, RMSSD, pNN50, absolute HF power, HF nu, SD1 and SD2, with a reciprocal rise in LF nu and in the LF/HF ratio — is concordant with that body of work, and the baseline absolute values (SDNN 48.59 ms, RMSSD 42.58 ms, LF/HF 0.90) lie within the range reported for healthy young adults in short-term supine recordings. The magnitude of the examination-related change in this cohort, in particular the near-doubling of the LF/HF ratio and the 52.8% fall in absolute HF power, is at the larger end of the published range; the timing of the second

recording, made within 48–72 hours of a high-stakes university examination rather than several days before it, is the most likely explanation. [Authors: insert here two to four sentences comparing these absolute values with two or three specific published studies — ideally Indian studies on medical undergraduates — once those papers have been retrieved and verified, noting whether the direction and magnitude of change agree with yours and explaining any discrepancy in terms of recording duration, timing of the pre-examination recording, breathing protocol or the anthropometric characteristics of the populations studied.]

The gender comparison in the present cohort yielded a nuanced picture. Boys and girls reported an almost identical degree of perceived stress before the examination and mounted an autonomic response that was qualitatively the same in direction and, for most indices, equivalent in magnitude. Girls preserved a significantly higher SDNN during the examination period and showed numerically higher vagal indices (RMSSD, pNN50 and SD1) that fell short of significance, while displaying a significantly faster heart rate; the normalised spectral powers and the LF/HF ratio were indistinguishable between the sexes. Sex differences in cardiac autonomic modulation are well documented: meta-analytic data indicate that women generally exhibit higher vagally mediated HRV and lower LF/HF ratios than men despite a higher resting heart rate, differences that have been attributed to the influence of oestrogen on central autonomic circuits and on baroreflex sensitivity, and to differences in cardiac size and in the distribution of vagal innervation.[13] The pattern seen here — higher heart rate together with preserved or higher variability in girls — is consistent with that literature. The considerable imbalance in the number of boys and girls in this batch, which reflects the actual composition of the intake, must be borne in mind when interpreting these comparisons: with 72 boys and only 28 girls the study had limited power to detect small between-sex differences, and the borderline result for pNN50 ($p = 0.056$) may well represent a type II error.

The significant correlation between the PSS-10 score and the vagal indices of HRV is a noteworthy observation. Subjective questionnaires and objective autonomic measures do not invariably agree, and the concordance found here suggests that in this population perceived stress was accompanied by a measurable physiological substrate rather than being purely a matter of self-report. It also raises the practical possibility that a brief, inexpensive five-minute recording could serve as an objective adjunct to questionnaire-based screening for students at risk.

The findings carry clear implications for medical education. Depressed HRV is an established independent predictor of adverse cardiovascular outcome and of all-cause mortality in longitudinal cohort studies[14], and reduced vagal tone has been linked to impaired emotional regulation, poorer executive function and diminished attentional control — precisely the faculties on which examination performance depends. A stress-induced autonomic shift may therefore be doubly disadvantageous, imposing both a long-term health cost and an immediate cognitive one. Interventions of demonstrated efficacy in restoring vagal tone, including slow-paced breathing and pranayama, yoga, mindfulness-based stress reduction, structured physical activity, adequate sleep hygiene and heart-rate-variability biofeedback, are inexpensive, safe and readily incorporated into the undergraduate timetable.[15,16] The introduction of the AETCOM module and of mentorship schemes in the current Indian undergraduate curriculum provides a natural framework within which such measures could be delivered.

Strengths of the study

The paired, self-controlled design eliminates between-subject confounding arising from genetic, anthropometric and constitutional differences in autonomic tone, since each participant served as his or her own control. Recording conditions were rigorously standardised with respect to time of day, ambient temperature, posture, respiratory rate, dietary and physical-activity restrictions and, in female participants, menstrual cycle phase. Objective electrophysiological measurement was combined with a validated subjective instrument, and analysis followed internationally accepted Task Force methodology in all three domains.

Limitations

- The study was conducted at a single centre on a single batch of students, which limits the external generalisability of the findings.
- Short-term five-minute recordings, though standard and well validated, cannot capture the circadian and ultradian information available from 24-hour Holter monitoring.
- Biochemical corroboration of the stress response — serum or salivary cortisol, plasma catecholamines or salivary alpha-amylase — was not undertaken, so the humoral limb of the response was not directly measured.
- Spontaneous rather than paced breathing was used; although respiratory rate was monitored and constrained, residual variation in tidal volume may influence high-frequency power.
- The marked numerical imbalance between male and female participants reduces the statistical power of the gender comparison.
- Sleep duration, dietary pattern, physical activity and the use of social media in the days preceding the examination were not quantified, and each may independently affect autonomic tone.
- No follow-up recording was made after the examination, so it is not known how rapidly, or whether completely, the autonomic indices returned to baseline.

Recommendations
Future work should include a post-examination recording to establish the reversibility and time course of recovery of the autonomic changes; concurrent measurement of salivary cortisol to corroborate the humoral limb of the stress response; a multicentric design with a more balanced gender distribution; and a randomised interventional arm evaluating whether a structured programme of slow-paced breathing, yoga or mindfulness training attenuates the examination-related fall in vagal tone.

CONCLUSION

Academic examination stress in first-year MBBS students was associated with a significant and consistent reduction in heart rate variability, with a disproportionate fall in the vagally mediated indices and a corresponding rise in the LF/HF ratio, indicating a shift of cardiac autonomic balance towards sympathetic predominance with parasympathetic withdrawal. These objective electrophysiological changes correlated with subjectively perceived stress measured by the Perceived Stress Scale. Since depressed heart rate variability is a recognised marker of increased long-term cardiovascular risk and of impaired emotional and attentional regulation, these findings argue for the routine screening of medical undergraduates for academic stress and for the formal incorporation of evidence-based stress-reduction measures, such as slow-paced breathing, yoga and mindfulness training, into the undergraduate medical curriculum. Heart rate variability analysis is simple, non-invasive and inexpensive, and is well suited to serve as an objective screening and monitoring tool in this setting.

DECLARATIONS

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

Source of funding: Nil.

Conflict of interest: None declared.

Data availability: The dataset analysed in the present study is available from the corresponding author on reasonable request.

Author contributions: [Authors: complete according to ICMJE criteria — for example: MFS and SB conceived the study, acquired the data and drafted the manuscript; AM and MBM designed the protocol and supervised the recordings; BMT analysed and interpreted the data; SM critically revised the manuscript for important intellectual content. All authors approved the final version and agree to be accountable for all aspects of the work.]

Acknowledgements: The authors thank the first-year MBBS students who participated in this study, and the technical staff of the Department of Physiology for their assistance with the recordings.

REFERENCES

1. Dyrbye LN, Thomas MR, Shanafelt TD. Systematic review of depression, anxiety, and other indicators of psychological distress among U.S. and Canadian medical students. Acad Med. 2006;81(4):354-73.
2. Thayer JF, Ahs F, Fredrikson M, Sollers JJ 3rd, Wager TD. A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. Neurosci Biobehav Rev. 2012;36(2):747-56.
3. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. Circulation. 1996;93(5):1043-65.
4. Shaffer F, Ginsberg JP. An overview of heart rate variability metrics and norms. Front Public Health. 2017;5:258.
5. Shaffer F, McCraty R, Zerr CL. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. Front Psychol. 2014;5:1040.
6. Tharion E, Parthasarathy S, Neelakantan N. Short-term heart rate variability measures in students during examinations. Natl Med J India. 2009;22(2):63-6.
7. Dimitriev DA, Saperova EV, Dimitriev AD. State anxiety and nonlinear dynamics of heart rate variability in students. PLoS One. 2016;11(1):e0146131.
8. Lucini D, Norbiato G, Clerici M, Pagani M. Hemodynamic and autonomic adjustments to real life stress conditions in humans. Hypertension. 2002;39(1):184-8.
9. Kim HG, Cheon EJ, Bai DS, Lee YH, Koo BH. Stress and heart rate variability: a meta-analysis and review of the literature. Psychiatry Investig. 2018;15(3):235-45.
10. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav. 1983;24(4):385-96.
11. Tarvainen MP, Niskanen JP, Lipponen JA, Ranta-Aho PO, Karjalainen PA. Kubios HRV - heart rate variability analysis software. Comput Methods Programs Biomed. 2014;113(1):210-20.
12. Thayer JF, Lane RD. A model of neurovisceral integration in emotion regulation and dysregulation. J Affect Disord. 2000;61(3):201-16.

13. Koenig J, Thayer JF. Sex differences in healthy human heart rate variability: a meta-analysis. Neurosci Biobehav Rev. 2016;64:288-310.
14. Tsuji H, Larson MG, Venditti FJ Jr, Manders ES, Evans JC, Feldman CL, et al. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. Circulation. 1996;94(11):2850-5.
15. Zope SA, Zope RA. Sudarshan kriya yoga: breathing for health. Int J Yoga. 2013;6(1):4-10.
16. Lehrer PM, Gevirtz R. Heart rate variability biofeedback: how and why does it work? Front Psychol. 2014;5:756.