

Short-term heart rate variability measures in students during examinations

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ABSTRACT

Background. Heart rate variability measures are reported to be altered in patients with various diseases, implying related abnormality of cardiac autonomic function. Could associated anxiety-induced mental stress, invariably present in all patients, contribute to these alterations? To address this question, we compared heart rate variability parameters computed from students during university examinations, a known cause of mental stress, with those computed during holidays when students are mentally relaxed. We felt that the changes in heart rate variability, if any, could be attributed to mental stress alone.

Methods. We compared short-term heart rate variability parameters computed from 5-minute electrocardiogram recordings during supine rest in 18 healthy students, along with recordings of respiratory rate and blood pressure, during university examinations and holidays.

Results. The mean of all the RR intervals was significantly lower, while the mean arterial pressure was significantly higher at the time of examinations. Measures of total variability, namely standard deviation of the normal-to-normal RR intervals and total spectral power, were significantly reduced during examinations. Low frequency normalized units (LF nu), high frequency (HF) nu and the LF/HF ratio were not significantly different.

Conclusion. The decreased mean RR interval indicates an overall increase in heart rate or cardiac sympatho-excitation during examinations. This is associated with a decrease in total heart rate variability at the time of mental stress. None of the parameters indicative of the ratio of the extent of modulation of cardiac sympathetic to parasympathetic activity were different. These findings may be useful when interpreting measures of heart rate variability in patients.

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INTRODUCTION

The resting autonomic control of the heart is reflected in the beat-to-beat fluctuations of the heart rate or the RR interval in the electrocardiogram (ECG). These variations in milliseconds of the duration of one cardiac cycle from the other is known as heart rate variability (HRV) and is traditionally expressed in statistical measures of time-domain analysis and as measures of spectral power under frequency-domain analysis. These measures are

reproducible when obtained under resting conditions,¹ implying constancy of the resting cardiac sympathetic and parasympathetic modulation.

Various studies have reported alterations in HRV parameters in disease conditions.^{2–4} The interpretation of this has been that the disease either causes, is caused by or is associated with abnormalities of cardiac autonomic control.

The diseased state is associated with underlying mental stress, not caused by the pathological process of the disease, but simply by anxiety invoked in patients due to awareness of the disease. Anxiety-induced stress is known to evoke the behavioural alerting response in humans, which is associated with an increase in sympathetic activity and a decrease in parasympathetic activity to the heart.⁵ This change in cardiac autonomic activity itself could alter the HRV measures in patients. The time of academic examinations is a known model of mental stress in students, as performance in examinations determines their future prospects. Several studies have reported changes in markers of stress in students during the time of examinations.^{6–8} We compared short-term HRV measures recorded during the time of university examinations with those recorded during holidays, in the same group of students. We thus studied the HRV measures during underlying mental stress alone in otherwise healthy volunteers.

METHODS

Eighteen students (18.7 [0.69] years; 9 males and 9 females) of a batch of 20 who volunteered to take part in the study were included. The study was approved by the Institutional Ethics Committee. After obtaining written consent, a 5-minute recording of the lead II ECG, following 10 minutes of supine rest, was obtained from the students on 2 different days, when they were under different emotional states. The first recording was done on the day of their university examination and the second was taken a month later when the same students were on holiday. There was no coercion to take part in the study and none of the authors were examiners in the university examination. The repeat recordings were made at the same time of the day, at similar room temperatures as the first, and 7 of the 9 female students were in the same phase of the menstrual cycle. On both days, all recordings were made a minimum of 2 hours after intake of food and 4 hours after consumption of caffeine-containing beverages. All the volunteers were healthy, non-smokers, were not on any medications and did not engage in any heavy physical activity for up to 24 hours before the recordings. The respiratory rate during the 5 minutes of ECG recording was noted and the blood pressure was measured by digital sphygmomanometry immediately after each ECG recording and the mean arterial pressure (MAP) calculated. The ECG was acquired using commercial leads and an amplifier (BIOPAC Systems, Inc., CA 93117, USA) and stored in a personal computer.

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No noise or ectopic beats were found on offline scrutiny of the ECG. RR interval time-series was computed from the ECG and subjected to short-term HRV analysis using a software (HRV Software version 1.1, Autonomic Function Laboratory, Department of Physiology, All India Institute of Medical Sciences, New Delhi), as per the recommendations of the Task Force.⁹ Mean of all the RR intervals (mean RR), standard deviation of the normal-to-normal RR intervals (SDNN), root mean square of successive differences between adjacent RR intervals (RMSSD) and the percentage of number of RR intervals with differences ≥ 50 ms (pNN50) were calculated in the time-domain. Frequency-domain measures were obtained by fast Fourier transformation and they included the absolute powers obtained by integrating the powers in the very low frequency (VLF) band of 0.0033–0.04 Hz, low frequency (LF) band of 0.04–0.15 Hz, high frequency (HF) band of 0.15–0.4 Hz, and the total power in all the 3 bands together. The normalized units (nu) of LF and HF power, as well as the LF/HF ratio, were considered.

The results, expressed as mean (SD), were analysed using the SPSS version 11.0 statistical software package and the Gaussian distribution of data was determined. Normally distributed data (mean RR, SDNN, LF nu, HF nu, respiratory rate and MAP) were tested with the paired *t*-test. Non-normally distributed data (pNN50, RMSSD, LF/HF ratio, VLF power, LF power, HF power and total power) were tested with the Wilcoxon signed rank test. A value of $p < 0.05$ was considered as significant.

RESULTS

The mean RR interval during the time of examinations was significantly reduced compared with that during holidays (Table I). The time-domain measures of SDNN and pNN50 were significantly reduced during the time of examinations as compared with holidays, but RMSSD showed no significant difference. The absolute power in the LF band and the total power were significantly less during examinations than during holidays. None of the other frequency-domain measures were significantly different. The mean breathing rate was the same across the two situations, but the MAP was significantly more during the time of examinations.

DISCUSSION

We compared short-term HRV measures computed during supine

rest in the presence and absence of mental stress. The anxiety associated with examinations is a source of mental stress that stimulates the sympathetic system of students and produces a state of cardiac sympatho-excitation. This is reflected in a significant decrease in the mean RR interval and a significant increase in the MAP on the day of university examinations.

The lowered mean RR interval denotes a higher overall resting heart rate during examinations. The mean RR interval is an indicator of the ratio of the cardiac sympathetic to parasympathetic tones (sympatho-vagal balance). The results suggest a tilt of the overall cardiac sympatho-vagal balance of the students towards the sympathetic side during examinations. In the supine position at rest during holidays, when the students are mentally relaxed, the sympathetic activity is expected to be least, and the ratio of cardiac sympatho-vagal tone the lowest.

The significant decrease in SDNN during the time of examinations reflects a decreased total HRV in the presence of mental stress. This is corroborated by the finding of a significant decrease in the total spectral power during examinations, which again points to a diminished total HRV under conditions of mental stress. The mean value of the LF, HF and VLF powers are of a lower magnitude during examinations, but the decrease is not statistically significant except in the case of LF power. However, the combined decrease in spectral power of all 3 bands contributes to the significant decrease in the total power during examinations. It is noteworthy that the time of underlying mental stress is associated with a significantly lower total HRV. Reduced HRV indicates diminished responsiveness of the cardiac autonomic system to normal physiological stimuli. Extrapolating this finding to the diseased state, it may be postulated that the mental stress associated with a disease could by itself decrease the total HRV, a fact that should be considered while interpreting the HRV in disease states.

Changes in the pNN50 and RMSSD are both reported in the literature to reflect HF changes in heart rate and therefore parasympathetic modulation.^{9,10} The finding of significant increase in pNN50 but not RMSSD during holidays is therefore contradictory. RMSSD is reported to be a better parameter than pNN50 to convey changes in resting HRV, as it is not affected by changes in the mean heart rate and is highly reproducible.^{9,10} Hence, the finding of no change in RMSSD may be more accurate and indicate that there was no change in the HF component of

TABLE I. Comparison of the short-term heart rate variability parameters, respiratory rate and mean arterial pressure (MAP) during the university examinations and holidays ($n=18$)

Parameters	During examinations	During holidays	p value
Mean RR (ms)	777.4 (114.3)*	867.3 (114)	0.036
SDNN (ms)	52.40 (21.672)*	74.20 (25.928)	0.012
pNN50	20.57 (19.038)†	39.37 (23.789)	0.028
RMSSD (ms)	49.99 (31.074)	74.03 (39.642)	0.070
VLF power (ms ²)	1294.11 (1263.204)	1886.89 (1582.61)	0.249
LF power (ms ²)	1192.09 (723.588)†	2154.89 (2157.405)	0.018
HF power (ms ²)	1690.64 (2096.269)	2891.89 (2622.458)	0.102
Total power (ms ²)	4176.8 (3385.3)†	6933.7 (4336.9)	0.025
LF nu	50.2 (16.7)	45.9 (20.1)	0.526
HF nu	49.8 (16.7)	54.1 (20.1)	0.526
LF/HF ratio	1.3 (0.8)	1.4 (1.8)	0.711
Respiratory rate (per min)	18.4 (3.6)	17.3 (3.3)	0.116
MAP (mmHg)	80.8 (10.6)*	76.3 (5.5)	0.033

All values are mean (SD) * $p < 0.05$ (paired *t*-test) † $p < 0.05$ (Wilcoxon signed rank test) mean RR mean of all the RR intervals
 SDNN standard deviation of the normal-to-normal RR intervals RMSSD root mean square of successive differences between
 adjacent RR intervals pNN50 percentage of number of RR intervals with differences ≥ 50 ms MAP mean arterial pressure
 VLF very low frequency LF low frequency HF high frequency nu normalized units

parasympathetic modulation. The spectral analysis finding of no significant change in the HF power across the two conditions further corroborates this reasoning.

Investigators have shown that modulation of both the sympathetic and parasympathetic cardiac inputs contributes to the LF power and modulation of only cardiac parasympathetic activity to the HF power.¹¹ However, investigations involving various manoeuvres have led to the association of LF power with sympathetic activity and HF power with parasympathetic activity.¹² Similarly, the LF/HF ratio has been used as a surrogate for the ratio of sympatho-vagal tone. To be more accurate, the LF/HF ratio is the ratio of the extent of fluctuations of the sympathetic tone to that of the parasympathetic tone.¹³ In our study the mean LF nu, HF nu and the LF/HF ratio showed no significant change across the two recordings. Thus, the parameters indicative of the relative proportion of the modulation of the two limbs of the cardiac autonomic system are the same under conditions of supine rest with and without underlying mental stress. Hence, it may be inferred that a change in these parameters in the diseased state is attributable to the disease process itself and not to the underlying mental stress.

The findings of previous studies on HRV during stress differ in some aspects from those of our study. This may be explained by the fact that the study group or design employed was different. Thus, an earlier study which compared the effect of examination stress on HRV parameters in students of a western population reports resting RR variance (RR variance is the square of SDNN) to be the same in students on the day of examinations and on a control day without any study schedule, but a significant increase in the resting LF nu and LF/HF ratio and a significant decrease in the HF nu on the examination day compared with that on the control day.⁸ Similar to our study, the mean RR was decreased while the arterial blood pressure was elevated on the examination day. The total spectral power, RMSSD and pNN50 were not compared. Also, the students had to answer a large number of questionnaires related to stress, coping and somatic symptoms, which could have made them conscious of the goal of the study, whereas in our study the students were not aware of the aim of the study.

In two other studies on HRV and stress, the LF nu and LF/HF ratio were found to be significantly increased in the group of subjects with higher levels of perceived stress.^{14,15} Unlike our study, the comparison was between two groups of subjects in both these studies. In one study the comparison was between healthy controls and subjects who were symptomatic and sought help from hospitals for chronic psychosocial stress.¹⁴ The design of this study excluded those with chronic psychosocial stress who were asymptomatic, which may have influenced the results. Moreover, the mean RR was surprisingly higher in the stress group than in the controls. In the other study, the comparison was between first-year undergraduate medical students who had higher scores of stress in a self-rated stress scale and those who had low stress scores.¹⁵ The basal resting heart rate was reported to be the same in the two groups but the resting cardiac autonomic modulations were found to be different.

A recent study reports reduced HF power and higher LF/HF ratio due to study stress in female medical technology students undergoing clinical training.¹⁶ In this study, the HRV measures computed from 24-hour ambulatory ECG, recorded before and during the clinical training, were compared. This is unlike our study where the HRV parameters were computed during supine rest.

Our study shows a clear reduction in both the time- and frequency-domain measures of total variability during examination stress, with no alteration in the relative contributions of the LF and HF modulations. Cardiac sympatho-excitation due to a physical stressor such as orthostatism is known to be associated with a reduction in total variability, along with alteration of the LF/HF ratio.⁹ In our study, the mental stress which produced cardiac sympatho-excitation was found to be associated with reduced total variability but no alteration in the LF/HF ratio. Such variations in observations of HRV indices has been the cause for much debate in the literature as to whether the HRV indices of 'sympatho-vagal balance' do reflect the autonomic activity in all conditions or not.^{17,18} Examination stress did not alter the LF/HF ratio in the students of our study, though the reduction in mean RR indicated a shift of the resting cardiac autonomic balance towards the sympathetic side.

We did not quantify the level of actual stress perceived or experienced by each student. Nevertheless, in as far as the aim of the study was to investigate a situation akin to disease conditions where the level of mental stress experienced by each patient varies from person to person, it was not necessary to quantify the stress.

In conclusion, we found that examination stress produced a significant decrease in the time- and frequency-domain measures of total HRV, but no significant difference in mean LF nu, HF nu and the LF/HF ratio. The results suggest that mental stress reduced the overall modulation and therefore responsiveness of cardiac autonomic control, without altering the relative proportion of modulation of the two limbs of the autonomic supply. These findings may help in the interpretation of HRV measures in patients, as the diseased state is also associated with mental stress, not pathologically linked to the disease but produced by the related anxiety.

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