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A STUDY OF STRESS AND AUTONOMIC NERVOUS FUNCTION IN FIRST YEAR UNDERGRADUATE MEDICAL STUDENTS

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Abstract : Psychological stress is a risk factor for hypertension and coronary artery disease. The purpose of this study was to assess the impact of real life stressor, that of stress among first year medical students on cardiac autonomic regulation. Stress levels in 36 non-smoking, healthy first year medical students of either gender were assessed on a self-rating scale. Cardiac autonomic regulation was tested using both conventional tests and spectral analysis of heart rate variability (HRV). Nine subjects who obtained scores on the stress scale in the upper quartile were classified as the "stress" group and the rest constituted the "no stress" group (n=27). There were no significant differences between the two groups on any of the conventional tests of autonomic nervous activity. The low frequency power in normalized units and low frequency high frequency ratio of heart rate variability in supine posture was significantly higher in the "stress" group compared to the "no stress" group. The low frequency power in normalized units was significantly positively correlated with total stress score. The changes were suggestive of a tilt in the resting cardiac autonomic balance towards increased sympathetic activity.

Key words : medical students stress autonomic functions spectral analysis heart rate variability

INTRODUCTION

Several studies both from the West (1-3) and from Asia (4) have reported that medical training is highly stressful particularly for those who are beginning their medical education. It is likely that the sources of stress are common across cultures (5). Studies have shown links between chronic life stress and cardiovascular disease (6). Psychological stress is a risk factor for hypertension (7) and coronary artery disease (CAD) (6). The physiological underpinning of this link may involve excessive

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sympathetic nervous system activation (6). It has been suggested that power spectral analysis of HRV might offer clues to the links between psychosocial risk factors and cardiovascular morbidity/ disease (8). Power spectral analysis of HRV reveals three spectral components: the very low (<0.04 Hz), low (0.04–0.15 Hz) and high frequency components (0.15-0.4 Hz) (9). High frequency power is largely a function of parasympathetic activity to the heart while low frequency component normalized for total power is used as a representative index of sympathetic activity to the heart (9).

The effect of stress on autonomic nervous function has most commonly been studied under laboratory conditions using standardized mental stress as the test stressor (10–13). These data, however, cannot be extrapolated to real life situations (7). Findings from a recent study that examined the effects of a real life stressor (university examination) on cardiovascular indices using spectral analysis of HRV in 30 healthy medical students were suggestive of cardiac sympathetic activation (7).

The aim of the present investigation was to test the impact of real life stressors among first year medical students on indices of resting cardiac autonomic regulation using spectral analysis of HRV and conventional autonomic tests.

METHODS

Thirty six non-smoking, healthy, first year medical students of either gender (male=19) were recruited into the study after obtaining written informed consent to a protocol that was approved by the institutional ethics review board. These students had already spent about six months in the medical school. A self-rating scale was used to assess levels of stress. The items in the scale were selected from a literature review of stress questionnaire used with medical students (14, 15). The questionnaire had 20 questions with five anchor points ranging from no stress (Score=0) to extremely stressful (Score=4). The items related to stress associated with academic demands, peer pressure, lack of time for personal needs and interpersonal relationships including those with teaching and administrative staff. The scale also included items generally reported by subjects in response to stress such as inability to sleep well, worrying, feeling tense and unhappy to name a few. The total score obtained on this questionnaire was used in further analysis.

Test and retest reliability of the scale was determined by administering the scale to 30 undergraduate medical students, who were not participating in the present study, a month apart. The test- retest reliability for the total score on the stress scale using Pearson's correlation coefficient was 0.72 significant at $P<.001$ and for the individual items it ranged from 0.42 ($P<.02$) to 0.74 ($P<.001$). We also examined the validity of the stress scale by comparing the total scores obtained on the stress scale against the General Health Questionnaire 28 item version (GHQ-28), which is a well-validated measure of psychological distress and has been widely used in India (16). A score of 5 or above on GHQ-28 identifies probable 'cases' that is, subjects with significant psychological distress. The 30 undergraduate medical students were divided into two groups (case vs. non-case) based on their GHQ scores and compared on the total scores on the stress scale. The mean stress scores at baseline (32.3±10) and a month later (31±11) was significantly different between the two groups (Baseline t = 2.49, df = 28, p = .01; Month later t = 3.68, df = 28, p = .001). Thus, the stress scale developed for the study had adequate reliability and validity.

Study of autonomic regulation

Subjects were studied in the morning after an overnight fast. After completion of the stress questionnaire the day before the testing, heart rate responses to the various maneuvers were measured after a mandatory 30-minute rest period. ECG was recorded continuously, immediately following each maneuver and subsequently until the heart rate had returned to normal. The subsequent test was performed only after the heart rate had returned to the resting level. Details of the various tests are as follows :

Cough : Subjects were asked to cough maximally once, which was repeated a second time after the heart rate had returned to basal values. The increment in heart rate was used as an index of vagally mediated withdrawal to the heart.

Deep breathing : The subjects were asked to breathe maximally at six breaths per minute. The maximum-minimum heart rate during each 10 s breathing cycle was measured and the highest difference during six successive breathing cycles was used in the analysis.

Maximum hand grip : The immediate heart rate response to a single maximal hand grip (Smedley's Dynamometer, TTM, Tokyo) sustained to a count of three was determined. The test was performed twice. The test is a measure of parasympathetic function.

Heart rate response to standing : The 30:15 ratio was calculated as the ratio of the longest RR interval around the 30th beat after standing up, to the shortest RR interval around the 15th beat during standing.

Valsalva maneuver : The subject was required to maintain a pressure of 40 mm Hg for 10 s after deep inspiration and after application of a nose clip. Valsalva ratio was calculated as the ratio of the longest RR interval within 20 beats of the maneuver, to the shortest RR interval during the maneuver.

Spectral analysis of heart rate variability

Details regarding ECG signal acquisition and power spectral analysis of heart rate time series have been described in our previous reports (26, 27). Briefly, the ECG was recorded continuously for a period of 10 minutes with subjects, supine and resting and for 10 minutes after subjects actively stood up. ECG signal was amplified through the AC-6016 module of the Nihon-Kohdon RM 6000 polygraph system (Nihon Kohdon, Tokyo, Japan) and a signal manifold, which digitized the signal using a CIO-AD Jr A/D card. The data were digitized online at 1000 Hz using an IBM compatible PC and the digitized signal was subjected to a spectral analysis after verifying the waveform characteristics using a specific software (CVM, World Precision Instruments Office, Sarasota, FL, USA). Data segments of 128 sec duration were sampled at 2 Hz to create 256-point datasets. For the duration of recording, data sets of 256 points constituting one segment, overlapping by half were processed. The linear trend was removed from each data set to avoid its contribution to low- frequency power. A Hanning window in the time domain was used to attenuate 'spectral leakage'. Spectral analysis was performed using a direct Fast Fourier Transform. The frequency resolution was 0.0078 Hz and the highest frequency evaluated was 0.4 Hz. The spectra obtained for the different data sets were averaged to reduce variance. Power was calculated in three bands, namely, very low frequency (VLF), low frequency (LF) and high frequency power (HF). The 0.04–0.15 Hz band is referred to as the LF power and the 0.15–0.4 Hz band is referred to as the HF power. LF and HF power were also expressed in normalized units, which represent the relative value of each power component in proportion to the total power minus the VLF component (15). We calculated the ratio of LF and HF power in normalized units as a reflection of sympathovagal activity. Subjects underwent all the autonomic tests on the same day and the order of the tests were kept constant for all the subjects throughout the study. Female subjects were studied on the 5th or the 6th day of the menstrual period.

Statistical analysis

Data are presented as mean±standard deviation (SD). Data were analyzed using SPSS for Windows (version 10.1). For the purpose of the analysis the subjects were divided into two groups : "stress" and "no stress" group. Those subjects obtaining scores on the stress scales in the upper quartile were classified as the "stress" group (n=9) and the rest constituted the "no stress" group (n=27). While traditionally, behavioral variables are treated as continuous dimensions on which individuals vary, other have argued that it is useful to study subjects as qualitatively different categories, as characteristics of a group defined in this manner tend to be more stable and has been particularly seen in experiments involving physiological responses to stress (28, 29). An independent 't' test was used to compare the various autonomic tests and measures of HRV between the "stress" and "no stress" group. The null hypothesis was rejected at $P<0.05$. We also examined the relationship between the total stress scores and various autonomic functions by using Pearson's correlation coefficient.

RESULTS

Table I depicts the demographic characteristics of the sample. The two groups were comparable in the baseline demographic characteristics (Table I). Males and females were equally distributed between the two groups with males constituting 56 % in the high stress group and 55% in the low stress group. 36 students who participated in the study did not differ from the rest of the class in academic performance in the university examination or in gender distribution. The composite stress score was normally distributed among the sample as determined by Kolmogorov Smirnov test (p=0.90) with the scores ranging from 0 to 65. Forty percent of the

TABLE I : Subject characteristics.

Variables	*High stress group (n=9)*	*Low stress group (n=27)*
Age (yr)	19.1±0.9	20.2±2.6
Height (cm)	160.8±9.8	165.1±11.2
Weight (kg)	56.5±10.5	60.4±10.4
BMI (kg/m^2)	21.8±2.8	22.0±2.3
Body fat (%)	22.2±7.8	21.1±6.2
Stress score	47.9±9.3	23.3±8.9
Basal heart rate	69.5±8.5	65.4±8.6

Values are mean±SD.

respondents found the academic demands of the course as moderately stressful and 21% extremely stressful. Respondents also reported that inability to find time to meet one's personal commitments (such as extra curricular activities or attending to the needs of a close relative) was stressful (16%). Less frequently reported were symptoms of overt psychological distress such as lack of sleep (3%), feeling depressed (5%) and inability to get along with others (5%). Group I (n=9) constituted the "stress" group with scores on the stress scale being in the upper quartile, while the group II (n=27; "no stress") constituted the lower three quartiles on the composite stress score.

There were no significant differences between the two groups on any of the conventional tests of the autonomic nervous activity (Table II). In contrast, the LF power in normalized units and LF/HF ratio of heart rate variability in supine posture was significantly higher in the "stress" group as opposed to the "no stress" group (Table III). Postural change in the form of active standing did not result in significant inter-group differences in any of the spectral measures of the heart rate variability. On Pearson's correlation coefficient, LF power in normalized units in supine posture was significantly positively correlated (r=0.34, p=0.04) with composite stress scores (Fig. 1). There were no correlation between stress scores and any of the other measures of autonomic functions.

TABLE II : Comparison of conventional tests of autonomic nervous activity between the two study groups.

Tests	*High stress group (n=9)*	*Low stress group (n=27)*
Delta HR cough	18.6±5.39	24.7±8.1
Delta HR MHG	20.5±5.9	24.8±9.4
Max delta HR during deep breathing	29.1±6.8	31.0±8.3
Delta change in heart rate during mental stress	6.5±5.0	4.7±2.9
Change of posture (30:15 ratio)	1.4±0.2	1.5±0.2
Valsalva ratio	1.7±0.2	1.7±0.3

Values are mean±SD.

TABLE III : Comparison of heart rate variability components between the two study groups in supine posture.

Heart rate variability components	*High stress group (n=9)*	*Low stress group (n=27)*
Total power (0.0-0.4 Hz)	2231.8±1390.5	4383.2±4352.5
Low frequency (LF) in absolute units (0.04-0.15 Hz)	1036.4±1085.9	1406.5±18.64.9
High frequency (HF) in absolute units (0.15-0.04 Hz)	586.0±437.6	1889.3±2111.7
LF in normalized units	61.1±15.8	47.4±17.8*
HF in normalized units	44.0±22.7	56.0±17.9
LF/HF ratio	2.8±3.4	1.2±1.1*

Values are mean±SD; *P<0.05.

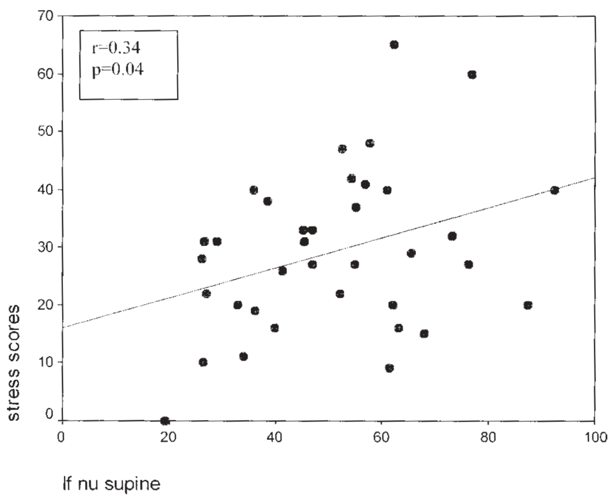

Fig. 1 : Relationship between low frequency power in normalized units and composite stress score.

DISCUSSION

In agreement with previous reports, majority of the students reported the academic demands of the medical training during the first year as a significant source of stress (1–3). It is also important to note that although 61% of the first year students reported academic demands as moderately to highly stressful, very few had symptoms suggestive of significant emotional distress such as lack of sleep or feeling depressed.

In the present study, the main finding is that in healthy young subjects, a real life stressor in the form of adjusting to the demands of medical training in 1st year undergraduate medical students, significantly impacts autonomic inputs of cardiovascular regulation. An earlier report, which examined the relationship between stress and cardiac autonomic function among medical students studied the effect of appearing in a university examination on cardiac autonomic indices (7). This study showed that during the stress day, the low frequency component in normalized units was increased and baroreflex gain was reduced. The authors interpreted the findings as suggestive of altered autonomic homeostasis with a shift towards cardiac sympathetic activation and vagal withdrawal. However, the nature of the stress was relatively acute. We chose to study the impact of adjusting to the demands of medical training among 1st year medical students, a stressor that is expected to affect the individual over a longer time frame.

There was a clear shift in the resting HRV indices characterized by a higher LF power in normalized units and a higher LF/HF ratio in subjects in the “stress” group, while there were no significant differences between the groups on any of the conventional autonomic tests. LF power in normalized units correlated positively with stress scores. This finding further confirms our earlier observations that spectral measures of HRV are more sensitive to subtle changes than traditional tests of autonomic function (22). The alterations in spectral measures of HRV suggest a tilt in the resting cardiac autonomic balance towards increased sympathetic activity. The LF/HF ratio is used as a marker of sympathovagal balance (30). In the present study, while the difference in HF power was not statistically significant different between the two groups, the “stress” group tended to have lower HF power in absolute units in resting conditions compared to the “no stress” group (p=0.07). The findings of higher sympathetic activity and possibly vagal withdrawal in the “stress” group is in agreement with an earlier study on the effect of acute stress in healthy young medical undergraduates (12). The changes in the autonomic cardiac indices were in the main, reflected by a significant change in LF power expressed in normalized units rather than absolute units. This finding extends earlier observations that normalization of spectral power may be important when studying effects of physiological maneuvers across groups (27).

This study has certain limitations. We have used only a single composite questionnaire-based measure of stress and have not studied psychological factors such as appraisal and coping mechanisms that may influence the stress response. The stress scores were obtained at only one point of time and hence the status of mental health of students prior to their entry to the medical course could have influenced the levels of stress. Other sources of stress such as familial or interpersonal problems were not examined. Biochemical parameters of stress such plasma or salivary cortisol was not measured. In addition, our data is restricted to cardiac autonomic activity; we did not evaluate vascular reactivity.

In conclusion, in medical students who obtained scores in the upper quartile of a self-report stress scale there was an impaired cardiac autonomic regulation represented by a higher LF power in normalized units and greater LF/HF ratio at rest. There was a significant correlation between stress scores and LF power in normalized units. The findings are suggestive of a shift in cardiac autonomic regulation towards sympathetic activation in response to real life stressors. However, these findings need to be replicated in a larger sample. It would be interesting in future studies to determine if interventions aimed at improving coping with stress, attenuate the changes in autonomic balance in young highly stressed individuals and whether these individuals are at increased risk of cardiovascular morbidity in later life.

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