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Impact of educational stress on cortisol, cardiac autonomic drive and academic performance of medical students

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ABSTRACT

Objectives: To assess stressors, heart-rate variability (HRV) and cortisol level in first year medical students (n-85), thrice in a year (beginning vs. mid-year vs. end-year). And we examined the association of these variables with their academic performance. **Methods:** Stressors were measured by medical student stressor questionnaire (MSSQ). HRV was assessed in eye-closed awake resting state for 5-min by Polar S810i. The salivary cortisol was assayed by ELISA method. Data were compared by Wilcoxon-Sign Rank test. Spearman correlation was applied between measured variables. Data are expressed as median (quartile 1-quartile 3), and significance was set at $p \leq 0.05$. **Results:** On MSSQ, students perceived mild to moderate degree of stress. About 8.24% and 11.8% of students perceived severe degree of stress in academic and inter-intrapersonal related stressors, respectively. Degree of stress in the beginning of the study was significantly high as compared to other visits. It was positively correlated with decreased HRV (decreased vagal activity and increased sympatho-excitation), high cortisol but with better academic performance in the beginning of the study. Cortisol (ng/ml) was significantly high in the beginning as compared to other visits [6.33 (5.05-7.43) vs. 1.33 (1.32-1.37) vs. 5.94 (5.1-6.6)]. The HRV measures showed mirror image of the cortisol among the visits. **Conclusion:** Stress level is mild to moderate degree in first year medical students which decreased as the year ended. Biochemical markers of stress, cortisol and HRV have similar trend.

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Key words: Heart rate variability, Cortisol, Academic stress, Academic performance, Medical students

INTRODUCTION

There are findings that stress elicits adaptive changes in the hypothalamic-pituitary-adrenal axis and the autonomic nervous system. Reports have shown that academic stress induces significant neuro-hormonal changes. It is indicated by increase in the plasma levels of Leptin, Neuropeptide Y, nitrite, nitrate, adrenomedullin, cortisol and ACTH.[1] It is also reported that medical students during their study encounter many stressors[2-4] which directly or indirectly affects their health and academic performance.

Stress influences the secretion of stress hormones, affects the autonomic drive of the heart and other accompanied responses. Heart rate variability (HRV) is considered to be one of the popular quantifiers of the autonomic drive to the heart than the reflex tests. It has been shown that decreased HRV is associated with worse prognosis in variety of diseases and disorders.[5] It is reduced in individuals reporting a greater frequency and duration of daily worry.[6] Moreover, decreased parasympathetic and increased sympathetic activity has been reported in students during clinical training as compared to before training.[7] Some studies have also suggested a linkage between autonomic and cognitive inflexibility[8,9] and have proposed that HRV could estimate the total activity of regulatory mechanisms in the students during their study.[10] We therefore aimed to study the impact of educational stress on cortisol, cardiac autonomic drive, and academic performance of the first year medical students.

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MATERIALS AND METHODS

Eighty-five medical (MBBS) students of aged between 18 and 28 years participated in this prospective study. The variables assessed were anthropometric (age and BMI), cardio-respiratory parameters, stressors, salivary cortisol level, and heart rate variability. Their annual academic performance was documented. The ethical approval was taken from the Institute Ethical Review Board and informed written consent was taken from all the participating students. All data were collected in afternoon between 3-5 PM. The study variables were assessed thrice in first year. First visit data was recorded after two months of their enrollment (November) in the institute, second was recorded after six months (May-June) of the first and the third visit (November) was recorded after six months of second visit.

A. Stressor assessment

The medical student stressor questionnaire[11] (MSSQ) was used to assess stressors and degree of stress in students. In MSSQ item number 4 consisting "quota system in examinations" was replaced by frequent examination, because there is not such system in our institute. Along with MSSQ, we assessed emotion and other related stressors (EOS) using a questionnaire consisting of ten items like staying far from home, financial crisis, food quality in mess, ill health, etc. It was graded as 0 = causing no stress at all to 4 = severe stress. Analysis of EOS was done by dividing total item score by ten, as there were ten items in it.

B. Heart rate variability assessment

In the eye-closed, awake state, the resting ECG at spontaneous respiration was recorded for 5 min in a semi-inclined posture on a comfortable dental chair. It was recorded in room temperature of 26±2°C. The Polar S810i and Polar Precision Performance SW (version-4.01.029) software was used for recording and analyzing HRV. The beat-to-beat raw data (i.e. RR intervals) were edited for any artifacts and subjected to fast Fourier transformation by using Kubios HRV (version 2.0) software, Kuopio University, Finland, for the power spectral analysis and processed according to the Task Force Guidelines, 1996.[12]

C. Saliva collection and cortisol estimation

Saliva was collected after rinsing mouth thoroughly with water 5-min prior to collection. Whole saliva was collected by unstimulated passive drool by allowing saliva to drip off the lower lip into a conical tipped plastic test tube. Saliva samples were frozen at or below –20°C within 30 min of collection. On the day of assay, samples were thawed to facilitate precipitation of mucins and then centrifuged at 3000 x g for ten min. The clear supernatant was used for cortisol estimation by DRG Salivary Cortisol ELISA (SLV-2930) and DRG Salivary Cortisol HS ELISA (SLV-4635) Kits.

D. Academic performance

Annual theory and practical examination marks were documented from the basic science disciplines (anatomy, biochemistry, microbiology, pathology, pharmacology and physiology). Marks were from three blocks (block 1- unit 0, block 2 - unit 1 and block 3 - unit 2), each block constituting of 400 maximum marks. Total score that can be secured by the students was 1200. Marks of the students were converted into percentage for uniformity. Following organ systems were distributed among the three blocks: block 1-basic-concepts, block 2 - genetics, growth, development, aging, and blood and immunity and block 3 - respiratory, cardiovascular and environment of above mentioned disciplines.

Statistical analysis

For comparison (three sets of data), Friedman followed by multiple comparisons (Wilcoxon-Sign Rank test) was done. Spearman correlation was applied among the test variables.

RESULTS

The anthropometric variables of the volunteers were displayed in Table 1. On comparing cardiovascular variables, heart rate was significantly high in first visits. Systolic (SBP) and diastolic blood pressures (DBP) were significantly high in first as compared to other visits (Table 2).

The marks obtained by the students in annual theory and practical examinations were 62.42% (56.92-67) and 66.58% (60.58-71.5), respectively. According to the institute examination policy, to succeed in the examination students must secure 50% marks in both theory and practical examinations. Out of 85, nine students failed.

There was significantly high cortisol in first visit as compared to other visits. And all the stressors were significantly high in first visit as compared to other visits. But the academic related stressor (ARS) was significantly high in second as compared to third visits (Table 3). In first visit, number of students perceiving high degree of stress in ARS, intra-inter personal (IPL), teaching and learning

Table 1: Anthropometric variables of the subjects, n-85

Variables	Mean±SD
Age (years)	20±1.766
Height (m)	1.67±0.097
Weight (Kg)	59.58±9.85
BMI (Kg/m^2)	21.45±2.52

(TLRS), group activities (GARS) and drive and desire related stressors (DDRS) were 8.24%, 11.8%, 2.4%, 4.8% and 2.4%, respectively.

The time domain parameters were significantly high in second (mid-year) as compared to other visits. In frequency analysis, high frequency (HF) power (ms^2), percent and n.u (normalized unit) were significantly increased in second as compared to other visits. And low frequency (LF) power percent was significantly decreased in second and third as compared to first visit. In addition, LF power n.u was significantly decreased in second as compared to other visits. In nonlinear analysis, SD1 and SD1/SD2 both were significantly increased in second as compared to other visits (Table 3).

There was no association of cortisol with stressors (MSSQ) among the visits. However, cortisol in first visit is positively associated with time and frequency measures of HRV (Table 4). And in second visit, cortisol is positively associated with few HRV measures: LF Hz and standard deviation of heart rate.

Table 5 displays the positive association of stressors with academic performance in first and second visits. In beginning, ARS and IPL stressors were positively associated with theory marks. Along with ARS and IPL, practical performance was positively associated with social related stressors (SRS) too. In mid-year, IPL and TLRS were positively associated with both theory and practical

Table 2: Comparison of cardio-respiratory variables among three visits, n-85

Variables	Median (q1-q3)			p1	p2	p3
	First visit	Second visit	Third visit			
Heart rate	72 (66-78)	67 (62-73)	71 (66-77)	<0.0001	0.029	0.001
Respiratory rate (min)	19 (16-20)	18 (16-20)	19 (16-21)	NS	NS	NS
SBP (mm Hg)	120 (110-122)	110 (110-120)	112 (108-120)	<0.0001	<0.0001	NS
DBP (mm Hg)	78 (70-80)	72 (70-80)	70 (65-80)	0.036	<0.0001	0.041

p1: p value on comparing first with second visits, p2: p value on comparing first with third visits and p3: p value on comparing second with third visits

Table 3: Comparison of all test variables among three visits, n-85

Variables	Median (q1-q3)			p1	p2	p3
	First visit	Second visits	Third visits			
Cortisol (ng/ml)	6.33 (5.05-7.43)	1.33 (1.32-1.37)	5.94 (5.1-6.6)	<0.0001	0.046	<0.0001
Stressors						
Academic	2 (1.5-2.54)	1.69 (1.23-2.07)	1.41 (1.07-1.91)	<0.0001	<0.0001	0.002
Intra and interpersonal	2 (1.57-2.43)	1.57 (1-2.28)	1.57 (1-2.14)	0.001	0.001	NS
Teaching and learning	1.6 (1.14-2)	1.14 (0.85-1.71)	1.14 (0.72-1.71)	<0.0001	<0.0001	NS
Social	1.33 (1-2)	1.16 (0.83-1.66)	1.15 (0.83-1.66)	<0.0001	<0.0001	NS
Drive and desire	1 (0.67-1.67)	1 (0.33-1.6)	1 (0.66-1.33)	<0.0001	<0.0001	NS
Group activities	1.5 (1-2)	1 (0.75-1.5)	1 (0.70-1.5)	<0.0001	<0.0001	NS
Emotion and other stressor	1.5 (0.9-2)	1.2 (0.9-1.7)	1.2 (0.9-1.5)	0.001	<0.0001	NS
Time domain measures of HRV						
Mean RR	820.1 (765.2-904.8)	899.7 (824.4-985.5)	848.6 (774.3-938.1)	<0.0001	0.002	0.001
SDNN	55.3 (42.3-62.8)	58.2 (47.1-75.7)	54.4 (43-69.2)	0.028	NS	NS
Mean HR	73.62 (66.41-79.1)	66.93 (61.43-73.31)	70.82 (64.22-78.27)	<0.0001	0.003	0.004
STD HR (standard deviation of heart rate)	4.65 (3.91-5.89)	4.36 (3.37-5.71)	4.48 (3.69-5.77)	0.045	0.027	NS
RMSSD	48.7 (34.5-67.3)	60.1 (40.8-74.3)	48.7 (34.9-67.4)	0.003	NS	0.008
NN50	115 (52-162)	135 (84-180)	112 (50-173)	0.003	NS	0.007
pNN50	32.2 (12.6-48.8)	41.4 (24.1-54.4)	31 (13.8-47.9)	<0.0001	NS	0.002
RR triangular index	11.94 (9.421-14.542)	13.76 (11-16.81)	12.61 (11.19-15.53)	<0.0001	0.006	NS
Frequency domain measures of HRV						
LF ms^2	650 (331-1079)	590 (306-1141)	564 (336-949)	NS	NS	NS
LF power percent	27.7 (19.5-34.4)	22.4 (16.1-31.8)	25 (17.2-32.6)	0.016	0.043	NS
LF power normalized unit (n.u)	41.2 (30-52.2)	32.2 (21.3-49)	37.1 (28.9-52.2)	<0.0001	NS	0.001
HF ms^2	872 (467-1737)	1200 (611-2415)	931 (483-1846)	0.002	NS	0.01
HF power percent	39.7 (26.9-54.3)	45.7 (34.7-62)	38.3 (25.5-53.4)	<0.0001	NS	<0.0001
HF power normalized unit (n.u)	58.8 (47.8-70)	68.4 (51-78.7)	62.9 (47.8-71.1)	<0.0001	NS	0.001
Total power ms^2	2714 (1669-3899)	2731 (1585-4841)	2679 (1708-4397)	NS	NS	NS
LF: HF ratio	0.7 (0.428-1.092)	0.474 (0.27-0.97)	0.59 (0.406-1.092)	<0.0001	NS	0.001
Nonlinear measures of HRV						
SD1	34.5 (24.4-47.6)	42.6 (28.9-52.6)	34.5 (24.7-47.7)	0.005	NS	0.008
SD2	66.3 (55.2-79.1)	71 (55.1-90.8)	64.7 (54.1-83.4)	NS	NS	NS
SD1:SD2	0.532 (0.45-0.62)	0.572 (0.46-0.76)	0.54 (0.44-0.63)	0.001	NS	0.005

p1: p value on comparing first visit with second visits, p2: p value on comparing first visit with third visits and p3: p value on comparing second visit with third visit

marks. There was no association of stressors with academic performance in third visits.

Table 6 displays the positive association of stressors with HRV measures only in first visit. ARS was positively associated with LF percent. Whereas, DDRS was negatively associated with HF percent and GARS was negatively associated with mean RR.

DISCUSSION

Stress via hypothalamus modifies the autonomic response and stress hormones (ACTH, Cortisol etc.) secretion for maintaining the body homeostatic condition. As educational stress elicits psychoneurohormonal changes and one of the natural models of stress, we conducted this study in first year medical students to resolve how stress associates with their academic performance.

Table 4: Relationship of cortisol and HRV measures among visits, n-85

Number of visits	Correlation of cortisol and HRV measures		
	HRV measures	Coefficient (r)	p
First	SDNN	0.333	0.0018
	STD HR	0.31	0.005
	RMSSD	0.26	0.018
	NN50	0.24	0.027
	RR triangular index	0.24	0.031
	TINN	0.26	0.0185
	LF power (ms^2)	0.37	0.0005
	HF power (ms^2)	0.26	0.019
	Total power (ms^2)	0.37	0.0006
Second	LF Hz	0.31	0.004
	STD HR	0.24	0.03
Third	Mean HR	0.216	0.047

Table 5: Relationship of stressors and academic performance, n-85

Visits	Stressors	Annual examination marks	r	p
First	Academic related stressor	Theory	0.25	0.02
		Practical	0.24	0.03
	Intra-interpersonal related stressor	Theory	0.27	0.02
		Practical	0.26	0.02
	Social related stressor	Practical	0.22	0.04
Second	Intra-interpersonal related stressor	Theory	0.25	0.02
		Practical	0.27	0.012
	Teaching and learning related stressor	Theory	0.26	0.02
		Practical	0.28	0.01

Table 6: Relationship of stressors and HRV measures in first visit, n-85

Stressors	HRV measures	r	p
Academic related stressor	LF percent	0.22	0.048
Drive and desire related stressor	HF percent	−0.22	0.046
Group activities related stressor	Mean RR	−0.29	0.009
	Mean HR	0.28	0.009

Our study demonstrates that there is association of educational stress with cardiac autonomic drive, cortisol and academic performance of the medical students. We found that the major sources of stress among students were academic related, intra-interpersonal related and teaching-learning related stress similar to other findings.[13-15] At the beginning of the course, students were found to be more stressed as compared to the mid-year and at the end of the year. It is contrary to the report where the degree of perceived stress faced by the students at the beginning of semester was less compared to the stress level experienced at the middle of the semester in pre-diploma science students.[4] Nevertheless, our results support our findings that students are more stressed in the beginning of course, as systolic and diastolic blood pressures (marker of sympathetic drive) were significantly decreased in second (mid-year) and third (end of year) visits as compared to the beginning of course. Moreover, the level of salivary cortisol was significantly decreased in middle and at the end of year as compared to the beginning of year. Similarly, the time domain parameters (SDNN, RMSSD, NN50 and pNN50) which are markers of parasympathetic activity were significantly increased in the mid-year as compared to other visits. It has been reported that low RMSSD is an indicator of stress either in chronic stress[16] or during stress induced by acute performance in the public area.[17] We found increased mean RR interval during middle of the year indicating an overall decrease in heart rate or cardiac sympatho-excitation (i.e. decreased stress). It is similar to the report where decreased mean RR in the examination is associated with the time of mental stress.[18]

Our results on HRV frequency analysis support our finding. At the mid-year, stress was decreased which was indicated by decreased sympathetic markers like LF power percent and LF power n.u as compared to the beginning and end of year. Similar result was reported in a study where stressed students have LF n.u higher than the "no stress" group.[19] In addition, in the mid-year, we found increase in HF: power (ms^2), percent and n.u (marker of parasympathetic activity) as compared to other visits. As well as, LF/HF ratio (sympatho-vagal balance) was significantly less in mid-year as compared to other visits. It is contrary to the report where it has been reported that LF/HF ratio was increased during the clinical training as compared to before clinical training in female medical technology students.[7] This might be due to the differences in course designed for MBBS and technology students. In our study, the non linear variables SD1 (vagal activity) and SD1/SD2 were also significantly increased in the mid-year as compared to other visits, showing better sympatho-vagal balance in the middle of course.

On comparing stressors of mid-year with end-year, almost all the stressors were same. The salivary cortisol level was increased at the end of year but did not reached to initial level. This shows that at the end-year they may be stressed and apprehensive due to the second year study exposure.

The interesting finding of our study is that in the beginning of the course, students having high academic related stress have high sympathetic activity (LF percent). In addition, students with high drive and desire related stress have lower parasympathetic activity (HF percent) and students with high group activities related stress have increased cardiac-sympatho activity (low mean RR). But there was no association of stressors and HRV in other visits. Overall, this shows that students were stressed in the beginning of course as compared to other visits with decreased HRV measures (increased sympathetic and decreased parasympathetic markers). It has been also shown that decreased HRV (decreased parasympathetic activity) serve as stress indicator in children.[16]

In our result, the beginning (ARS, IPL and SRS) and mid-year stressors (IPL and TLRS) were positively associated with academic performance. It is contrast to the study where it has been reported that there is a significant correlation between the perceived degree of stress and academic performance at the end of semester.[4] We found students having moderate degree of stress performed better in the annual examinations. Therefore, these stressors seemed to be favorable for students in securing better marks in the examination. However, we did not found correlation of cortisol and stressors in any visit. This might be due to moderate degree of stress perceived by the students. Stress is coped by the students without significant increase in cortisol level. In the mid-year, the positive association of cortisol and LF Hz shows that students having less stress have low cortisol and vice-versa.

The shortcomings of the study are that HRV and cortisol estimation during their examination time could not be done. The seasonal variation might have influenced end year cortisol and HRV measures. However, cortisol level was significantly different among the visits. Therefore, there is no effect of season on our results. And there are reports mentioning absence of seasonal variation of cortisol in healthy individuals, supporting our results.[20, 21]

The strength of the study is that it is a prospective study and has established HRV measures as one of the markers of stress, which can be seen even at moderate degree of stress. Stress markers, cortisol and HRV measures showed similar trend with stress being high in the beginning, decrease in the mid-year and increase at the end of year.

CONCLUSION

Stress in medical students is of moderate degree and positively associated with decreased cardiac autonomic drive (HRV) in the beginning of course. But moderate degree of stress favored their academic performance. Cortisol and HRV measures have similar trend of changes during stress. Thus, decreased HRV can be one of the stress markers which can be assessed by noninvasive method.

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Authors Contribution:
NU – conceived, designed and carried out the study, participated in the sequence alignment, drafted and finalized the manuscript. **RK –** participated in research design and worked in questionnaire designing and implementing. **BHP –** participated in the sequence alignment. Helped in the design of the study and involved in writing and reviewing the manuscript.

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