



plasma catecholamine and corticosteroids. Stressors can be physical conditions such as heat or inflammation, exercise, etc. or psychological like exam, interview, etc. Acute stresses improve the performance by increasing sympathetic discharge for a short time but chronic stress increases sympathetic discharge for longer time. Sympathetic over activity for longer time is known to be associated with hypertension and increase in cardiovascular morbidity and mortality. The changes produced by physical stress such as effect of exercise on various physiological parameters are well studied but impacts of psychological stressors are less studied. The studies so far on psychological (1, 2) stresses are largely restricted to laboratory using questionnaire. The studies on real life stressor are also very limited. Several studies both from West (3-6) and from Asia have reported that medical training is highly stressful particularly for those who are beginning their medical education. It is likely that sources of stress are common across culture (7). Studies have shown link between stress and cardiovascular disease (8). Psychological stress is a risk factor for hypertension and coronary artery disease. Its physiological mechanism may involve excessive sympathetic activation (9). It has been suggested that power spectral analysis of heart rate variability (HRV) might offer clue to links between psychosocial risk factors and cardiovascular morbidity (10). Power spectral analysis reveals three spectral components: The very low frequency (VLF) (<0.004 Hz), low frequency LF (0.004-0.15 Hz) and high frequency HF (0.15-0.4 Hz). HF is largely a function of parasympathetic activity to heart while LF component normalized for total power is used as a representative index of sympathetic activity to the heart (11).

There are many stress busters in today's era but the ancient and most balanced stress relieving cum autonomic stability achieving method is yoga. In yoga, Pranayama is known to modulate autonomic output. The combination of various types of pranayama helps in achieving and maintaining autonomic balance between two components (sympathetic and parasympathetic of autonomic nervous system. Pranayama (12) forms fourth limb of classical Patanjali's Ashtanga yoga (eight fold sadhana).

From literature it appears that stress either physical or mental, leads to cardiovascular morbidity. Newly admitted medical students are likely to be exposed to various stresses like change of environment, demanding medical education and different teaching protocol in a medical college. Pranayama is known since ancient times to relieve stress and stabilize autonomic function of the body. Hence it was decided to study the effect of Pranayama on first M.B.B.S newly admitted medical students by comparing certain parameters.

#### MATERIALS AND METHODS

The subjects were first M.B.B.S students and the sample size was 59 consisting of 27 males and 32 females who had joined their first M.B.B.S in July and enrolled for the study within one month after joining first M.B.B.S. The protocol was approved by ethical committee of the Institute. The students were recruited in the study, then history taking and clinical examination was done keeping in mind following inclusion and exclusion criteria.

#### **Inclusion criteria**

First M.B.B.S newly admitted students

to the medical college of comparable socioeconomic status, psychosocial nature and dietary habit. They should voluntarily participate and undergo Pranayama training every evening for one hour as taught by the instructor.

#### **Exclusion criteria**

They should not be practicing any known stress relieving or relaxation technique. They should not be having any drugs or beverages in quantity which affect the autonomic nervous system like anticholinergic drugs. They should not be having any major illness which is known to affect the autonomic nervous system.

The group of students thus selected were briefed about the study. After the orientation session, informed written consent was taken, stress questionnaire was put. The pretested and prevalidated stress questionnaire of questions was used. The scoring system has 5 points starting from 1 i.e. almost never to 5 i.e. most of the time. The maximum possible score was 5 and minimum possible score was 1 for any given question. The subjects were instructed to mark under only one point and attempt all the questions. The autonomic function tests were done. This was followed by practice of Pranayama (10) for 2 months, 1 hour/day for 5 days/week and again stress questionnaire was put and the autonomic function tests were performed on the study group. The Pranayama practiced were Kapalabhati, External Kumbhaka (Bahya), Easy Comfortable Pranayama (Sukha Purvaka), Surya Bhedan, Ujjayi, Sitkari and Sitali. The subjects underwent all the autonomic function tests on the same day and the order of tests was kept constant for all the subjects throughout the study. The autonomic function tests in female

subjects were done on 5th or 6th day of the menstrual cycle.

Following Autonomic Function Test was done using standard procedure (11, 13, 14, 15).

The cardiovascular autonomic tone in the mentioned study was heart rate variability (HRV). HRV is beat to beat variation in heart rate (i.e. intervals) under resting condition. These beat to beat variations occur due to continuous changes in the sympathetic and parasympathetic outflow to the heart. Though HRV can be measured over any length of time of recorded ECG, as per Task force Guidelines (1996) at least 5 minutes of ECG must be recorded to quantify sympathetic and parasympathetic tone. The details of recordings are elaborated as under.

- 1) *Procedure*: Subject preparation: Following instructions are given to the subjects: (i) To avoid food preceding 2 hours of the testing, (ii) No coffee, nicotine or alcohol 24 hours prior to testing, (iii) Drugs known to affect cardiovascular autonomic functions like anticholinergic (including antidepressants, antihistaminics and over the counter cough and cold medications) for 2 days prior to testing, (iv) To wear loose and comfortable clothing.

Software used was Vagus HRV designed by RMS Chandigarh and hardware compatible with software for acquisition of ECG i.e. leads and digitalization by analog to digital converter (ADC) was provided by the same company. The software was designed according to Task force Guidelines for technical requirements and recommendations, algorithm standards

and recommendation. The reproducibility and standardization of equipment were tested before beginning the study.

2) *Recording*: For short term analysis of HRV, ECG is recorded in lead I in supine position for 5 minutes after 15 minutes of supine rest on a bed. Room temperature was around 24 degree Celsius. Subject was instructed to close the eyes and to avoid talking, to avoid moving hands, legs or body, to avoid coughing during test and to avoid sleeping.

1) *Acquisition*: The ECG signal continuously amplified, digitalized and stored in the computer for offline analysis.

2) *Analysis*: The detection of R wave is done by software using tall peak detection algorithm. Then it computes RR intervals after the R wave detection. RR intervals in millisecond are plotted against time in seconds to obtain tachogram.

3) *Quantification of HRV*: The analysis of HRV is done by the two methods: the time domain, and frequency domain. In above study, time domain and frequency domain parameters were analyzed which are described as under.

*Time domain methods*: **SDNN**-Standard deviation of the RR intervals, **SDSD**-Standard deviation of differences between adjacent RR intervals, **RMSSD**. The root square of the mean of the squares of differences between adjacent RR intervals, **NN50**-Number RR interval differences=50 ms and **pNN50**-Percentage of NN50.

RESULTS

Table I below shows pre and post intervention answer to questionnaire used to measure subjective stress level in the study group.

Power spectral band are calculated in ms<sup>2</sup>

TABLE I

Particular	Pre Mean±SD (n=59)	Post Mean±SD (n=59)	WSRT	P Value
Do you get on well with your coworker?	4.42±0.86	4.61±0.56	1.73	0.083
Do you let others know how are you feeling?	3.17±1.04	3.46±1.13	1.83	0.067
Do you suffer from constipation or diarrhea?	1.83±0.93	1.47±0.68	3.23	0.001
Do you get jealous of others?	1.93±0.91	1.69±0.91	2.28	0.022
How often do you catch cold?	3.10±1.23	2.90±1.21	1.25	0.21
Do you crave sweet things to eat?	3.19±1.09	3.12±1.10	0.69	0.49
How often do you suffer from headache?	2.76±1.09	2.42±1.09	2.48	0.013
When you are ill does it take long to get over it?	2.31±1	2.19±1.04	1.02	0.31
Are you quick to anger?	3.03±1.33	2.66±1.24	2.21	0.027
Do you feel you are under too much pressure?	3.31±0.95	2.83±1.02	3.25	0.001
Do you feel refreshed at beginning of the day?	3.92±1	3.90±1.16	0.27	0.79
How often do you feel lonely?	2.83±1.13	2.53±1.07	2.23	0.03
Do you drink alcohol?	1±0	1.02±0.13	1	0.32
Does your heart pound?	2.75±0.96	2.34±1.04	3.01	0.003
Do you suffer with difficulty in sleeping?	1.88±1.13	1.66±0.90	1.91	0.05
When conflicts do you overreact?	2.39±1.16	2.19±0.86	1.15	0.25
Do you have difficulty in concentrating?	3.37±0.93	2.90±1.11	3.37	0.001
Do you have allergy flare up?	1.80±1.01	1.47±0.82	2.65	0.008
Do you sweat excessively?	2.27±1.22	2.08±1.18	2.29	0.02
Are you happy?	4.02±0.94	4.39±0.79	3.84	0.00001

(absolute power) and in normalized power (n.u.). For example to normalize unit of LF it is calculated by the formula: (LF/total power-VLF)  $\times$  100. The normalization emphasizes the controlled and balanced behavior of 2 parts of autonomic nervous system.

TABLE II: Analysis of total stress score and various tests for autonomic function.

Particular	Pre Mean $\pm$ SD (n=59)	Post Mean $\pm$ SD (n=59)	P Value
Total score	52.24 $\pm$ 6.14	49.17 $\pm$ 5.90	0.0001
Max. RR int.	0.93 $\pm$ 0.12	0.95 $\pm$ 0.15	0.29
Min. RR int.	0.57 $\pm$ 0.08	0.56 $\pm$ 0.08	0.43
Mean RR int.	0.77 $\pm$ 0.09	0.79 $\pm$ 0.18	0.37
Max./Min. RR int.	1.65 $\pm$ 0.27	1.70 $\pm$ 0.30	0.24
Max. HR	107.15 $\pm$ 13.67	109.61 $\pm$ 17.10	0.30
Min. HR	65.37 $\pm$ 8.47	64.63 $\pm$ 8.49	0.55
Mean HR	78.83 $\pm$ 10.10	79.86 $\pm$ 9.75	0.44
SDNN	61.51 $\pm$ 27.35	66.40 $\pm$ 27.34	0.19
RMSSD	67.74 $\pm$ 33.98	76.38 $\pm$ 32.02	0.09
NN50	109.54 $\pm$ 66.86	126.71 $\pm$ 73.58	0.08
pNN50	30.41 $\pm$ 17.75	33.51 $\pm$ 20.03	0.23
LF in n.u.	63.27 $\pm$ 12.88	53.03 $\pm$ 13.58	0.0001
HF in n.u.	36.79 $\pm$ 12.88	46.95 $\pm$ 13.56	0.0001
LF/HF	2.10 $\pm$ 1.19	1.32 $\pm$ 0.71	0.0001
VLF	66.80 $\pm$ 23.39	58.01 $\pm$ 24.33	0.005

TABLE III: Correlation between total stress score and various parameters for autonomic functions.

Correlation between	r value (pre)	r value (post)	p value (pre)	p value (post)
Total score and LF in n.u.	0.42	0.24	0.0001	0.045
Total score and HF in n.u.	-0.42	-0.24	0.0002	0.045
Total score and LF/HF in n.u.	0.36	0.22	0.01	0.06

{n.u.-normalized power, Max. RR int. - maximum RR interval, Min. RR int. - minimum RR interval, Mean. RR int.- mean RR interval, Max./Min. RR int. - maximum divided by minimum RR interval, Max. HR maximum heart rate, Min. HR - minimum

heart rate, Mean HR - mean heart rate, SDNN - Standard deviation of the RR intervals, RMSSD - The root square of the mean of the squares of differences between adjacent RR intervals, NN50 - Number RR interval differences=50 ms, pNN50 - Percentage of NN50, LF in n.u. - Low Frequency (LF), HF in n.u. - High Frequency (HF), LF/HF - Low Frequency (LF)/High Frequency (HF), VLF - Very low Frequency (VLF)}

## DISCUSSION

It has been reported that pranayama improves cardio respiratory functions (16, 17) and alters autonomic functions. There are studies on effect of particular pranayama on autonomic nervous system like effects of nadishuddi and Kapalabhati. Effect of shavasan on RR interval variation (RRIV), deep breathing difference (DBD), and heart rate, blood pressure & rate-pressure-product (RPP) response to CPT were measured before and after was studied by Madanmohan, Udupa K, Bhavanani AB, Krishnamurthy N, Pal GK. Significant blunting of cold pressor-induced increase in heart rate, blood pressure and RPP by shavasan was seen during and even five minutes after CPT suggesting that shavasan reduces the load on the heart by blunting the sympathetic response( 18). Pranayama breathing has been shown to alter the autonomic activity. Telles et al have demonstrated pranayama breathing through right nostril results in increase in sympathetic activity whereas left nostril breathing reduces it (19, 20). Raghuraj et al (21) have reported that slow pranayama (nadishuddi) increase parasympathetic activity whereas fast pranayama (Kapal Bhati) increase sympathetic activity. Pal et al (22) studied the effect of short term

breathing exercise on autonomic function. They have studied kapalbhati and nadishuddhi on Valsalva manuvre, deep breathing and lying to standing. Since Udupa et al (16, 17) has reported that fast and slow pranayama have synergistic effects, we clubbed fast and slow pranayama in the present study. The effect of combination of pranayama, described earlier was studied on various autonomic function tests. The earlier studies along the same lines were done on small number of subject whereas our study has taken larger sample size and therefore the results obtained can be used for extrapolation at large.

The present study was conducted on 59 first M.B.B.S students (29 males and 32 females) newly admitted to belonging to age group 17-22 years. In the present study, the main finding is that in healthy young subjects, a real life stressor in form of adjusting to the demands of medical training in first years, significantly impacts autonomic inputs of cardiovascular regulations after practicing pranayama.

The stress level was tested using stress questionnaire and Wilcoxon sign rank test used to test the significance. Twelve questions showed significant change in response after pranayama which show subjective improvement in perception of stress. The psychological change improves the physiology of body according emerging aspect of medicine which is known as mind-body medicine or psychoimmunoneurology.

In our study of HRV, only frequency domain parameters showed significant change because for short term HRV, frequency domain parameters reflect autonomic

function. Time domain parameters are better commented on long term or 24 hours ECG acquisitions. In frequency domain parameters we observed the significant decrease in the power VLF. VLF indicates possibly renin angiotensin system. Renin angiotensin system is activated by sympathetic, decrease in BP and decreased sodium delivery to macula densa cells. The possible decrease VLF can be attributed to decrease in sympathetic stimulation. LF in n.u has decreased which indicated that sympathetic influence has decreased. HF in n.u has increased which indicated that parasympathetic influence has increased. LF/HF is also showing significant reduction of ratio indicating improvement of Sympathovagal balance i.e. decrease in sympathetic influence and increase in parasympathetic influence.

There is positive correlation between total score and LF in n.u. Total score and LF/HF ratio before pranayama. Also, after pranayama there is positive correlation between total score and LF in n.u and there is negative correlation between total score and HF in n.u. since most of the questions were negative, there exist positive correlation with LF power and negative correlation with HF power but the significant correlation points out that questionnaire i.e. test for stress and autonomic functions have correlation. Such correlation is also studied by Srinivasan et al (22). According to mind body medicine and psychoimmunoneurology, we use thought process of the patient to bring about self healing.

Jerath et al (24) has explained the mechanism of how pranayamic breathing interacts with the nervous system affecting metabolism and autonomic functions remains

to be clearly understood. It is their hypothesis that voluntary slow deep breathing functionally resets the autonomic nervous system through stretch-induced inhibitory signals and hyperpolarization currents propagated through both neural and non-neural tissue which synchronizes neural elements in the heart, lungs, limbic system and cortex. During inspiration, stretching of lung tissue produces inhibitory signals by action of slowly adapting stretch receptors (SARs) and hyperpolarization current by action of fibroblasts. Both inhibitory impulses and hyperpolarization current are known to synchronize neural elements leading to the modulation of the nervous system and decreased metabolic activity indicative of the parasympathetic state. In this paper authors proposed that pranayama's physiologic mechanism through a cellular and systems level perspective, involving both neural and non-neural elements.

There are a few limitations in our study. We have used only a single composite questionnaire based measure of stress and have not studied psychological factors such as appraisal and coping mechanism that influence stress response. Other sources of stress such as familial, personal etc were not assessed. Biochemical parameter of stress such as plasma or salivary cortisol was not measured. In addition, our data is restricted to cardiac autonomic reactivity and did not evaluate vascular reactivity. For any

confounding factor even control group having similar features but not practicing pranayama was not considered.

The scope of the present study can be expanded by further studies. This is exploratory study. A further study has to be designed taking advantage of current molecular methods and imaging technology to study various biochemical and physiological parameters. This will not only enable to fortify cause and effect relationship but also throw light on the underlying mechanism.

Thus, the stress level has reduced after 2 months of practicing various pranayama as evident by decrease in total stress score which is highly significant. Therefore pranayama improves subjective perception of an individual. The autonomic outflow to heart improves as indicated by increase in HF and decrease in LF. Therefore Pranayama increases parasympathetic outflow and decreases sympathetic outflow. LF/HF ratio reduced significantly after 2 months of practice of pranayama indicating a better sympatho vagal balance with resting balance tilting toward better parasympathetic control.

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