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Original Article

Heart rate variability changes during stroop color and word test among genders

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Abstract

Stress is the reaction of the body to a change that requires physical, mental or emotional adjustments. Individual differences in stress reactivity are a potentially important risk factor for gender-specific health problems in men and women. The Autonomic regulation of the cardiovascular system is most commonly affected by stress and is assessed by means of short term heart rate variability (HRV).The present study was undertaken to investigate the difference in the cardiovascular Autonomic Nervous System response to mental stress between the genders using HRV as tool. We compared the mean RR interval, Blood pressure and indices of HRV during the StroopColor Word Test (SCWT).Twenty five male (Age19.52±0.714, BMI 22.73±2 kg/m^2) and twenty five female subjects (Age 19.80±0.65, BMI 22.39±1.9) performed SCWT for five minutes. Blood Pressure (SBP $p<0.01$, DBP $p<0.042$) & Mean HR ($p<0.010$) values showed statistically significant difference among the genders. HRV indices like LFms^2 ($p<0.051$), HF nu ($p<0.029$) and LF/HF ratio ($p<0.025$, $p<0.052$) show statistically significant difference among the genders. The response by the cardiovascular system to a simple mental stressor exhibits difference among the genders.

Introduction

Stress is an inevitable part of everyday life. Physiological stress affects homeostasis, leading to increase in the production of cortisol by the hypothalamic-pituitary adrenal axis (HPAA) and changes in the cardiovascular system brought about by the Autonomic Nervous System (ANS) (1). On the other hand, psychological stress causes bodily changes that originate in the higher centres of the brain and act in the periphery via the ANS (2). The autonomic response to stress has been assessed using different techniques. Plasma and urinary catecholamine levels, cardiac norepinephrine spillover and microneurographic techniques are some of the methods that have been popularly used over the years. All the aforementioned techniques do not provide any information about the activity of the parasympathetic branch of the ANS. The concept of Heart Rate Variability (HRV) has been proposed as a robust and sensitive tool to study the influence of both sympathetic and parasympathetic systems on the heart (3). Currently, the time and spectral analysis of HRV is seen as a research and clinical tool for the study of cardiovascular autonomic

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regulation (4). Short term HRV has been used as a quantitative probe to assess the modulation of heart rate by the Sympathetic Nervous System (SNS) and the Parasympathetic Nervous System (3, 5).

Numerous stressors have been developed to study the autonomic modulation of cardiovascular function in the laboratory. These include physical (e.g. stress ergonometry, exercise, cold stress test or Valsalva manoeuvre) and mental stressors (5, 14). The hemodynamic response to mental stress differs from the corresponding response to physical exercise. Exercise causes cardiovascular changes in accordance with the metabolic demands of motor behavior. On the other hand, mental stressors do not cause an activity proportionate increase in energy demands and may be regarded as metabolically exaggerated (6, 7). Such a large magnitude cardiovascular reaction to mental stress is believed to be an important mechanism underlying the pathophysiology of Coronary Artery Disease (8, 9, 10). Mental stress has also been shown to decrease the Parasympathetic modulation of heart rate (11, 12). This in turn could be a cause for dangerous rhythm disturbances in Post MI patients (13).

Stroop Color Word test (SCWT) is commonly used as a mental stressor in cardiovascular research (14) it consists of three components : quick reading, identifying colors and interference (15). The Stroop test was originally introduced by John Ridley Stroop as a series of cards presenting congruent and incongruent stimuli. Essentially it comprises of three kinds of stimuli : colored rectangles serving as the control, names of colors written in the congruent color (eg. 'GREEN' in green) and the names of colors presented in an incongruent color (GREEN' in red). The test requires the subjects to name the color of the rectangles, the words of the congruent stimuli and the color of the incongruent stimuli, without reading the actual word itself (16). When presented with the incongruent stimuli, the subject gets into a conflict filled stressful situation because his reply is influenced by his learning (the tendency to read the word and not name the color). This is referred to as the Stroop interference (14, 17). Among all mental stressors, only the Stroop test has been found to activate all components of the SNS (18). Fauvel et al concluded that this test could also be useful in epidemiological studies, as well as in therapeutic assessment of blood pressure and heart rate changes under mental stress conditions (19). We used the computerized version of this test wherein individual color words appear sequentially on the screen. Studies have shown that the computerized Stroop test causes an increase in heart rate, decreases HRV and increases catecholamine secretion thus making it a suitable and well standardized mental stressor (20, 21, 22, 29).

Gender plays an important role in health and disease. Males and females respond differently to mental stressors. Kudielka et al and Kajantie et al showed that adult men exhibited greater HPAA and sympathetic response to laboratory stressors (23, 24). In women, the stress response depends on the phase of menstrual cycle, menopausal status and pregnancy (25). Estrogen in women has been shown to buffer the sympathetic and HPAA arousal thereby reducing the incidence of cardiovascular disease. (26). The cardiovascular reactivity to stress is associated with changes in HRV parameters and again it is age dependent. A recent Brazilian study noted a significant increase in heart rate and Systolic Arterial Pressure during a mental stress test in young women aged 22-27 years (27).

However, there is a paucity of literature comparing the stress response to Stroop test, among genders in young adults. Hence, our study aims to compare the HRV changes in response to the Stroop Color Word Test, among the genders.

AIM

To assess the influence of a Laboratory stressor (StroopColor Word Test) on Heart Rate Variability parameters and blood pressure.

To compare the autonomic response to stress between the two genders.

Materials and Methods

Fifty healthy young volunteers (25 male and 25 female) were included in the study. They were divided into two groups: male and female after matching for age and BMI. (Age 20±2 years, BMI 19-25). All female subjects were in the Luteal phase of the menstrual cycle (23). None of the subjects had been previously diagnosed with cardiovascular or psychiatric disorders, nor were they taking any medication that would alter their heart rate. Subjects who had taken a Stroop Test earlier and those who were color blind or dyslexic were excluded from the study. Volunteers were instructed not to eat a heavy meal, ingest caffeine or alcohol or exercise 4 hours before arriving at the laboratory. They were also asked to void urine before the test. The experimental protocol was approved by the institutional ethical committee, Government Stanley Medical College and Hospital, Chennai, India. An explanation of experimental protocol was provided and formal written consent was obtained from each subject.

On arrival at the laboratory, baseline blood pressure was recorded for all subjects using a sphygmomanometer. The computerized version of the SCWT was used as a mental stressor and the subjects were trained to respond using a keyboard. Subjects then underwent a familiarization protocol of the computerized SCWT with 12 practice trials. They were instructed to answer as quickly and accurately as possible. Following this, ECG electrodes and respiratory transducers were applied to the subjects and connected to the RMS polyrite to record the lead II ECG and respiratory excursions (13). Subjects were seated throughout the testing. The experiment was carried out in three phases - Pre stress, stress and recovery phases. During the *pre stress phase*, the subjects were instructed to sit with their eyes closed, in a quiet and relaxed atmosphere for ten minutes. This was followed by the application of the experimental stressor, the computerized version of the StroopColor Word test, for a period of 5 minutes. This test entailed the individual presentation of cues (2 cm in height), in the centre of a computer monitor on white background. Cues consisted of color-words (red, green, blue and black) and colored rectangles (neutral stimuli) presented in random order. The words were presented either in the color itself or in an incongruent color. The subjects were asked to indicate the color of the rectangle, and the color of the word, without reading the actual word itself. Key press was used as the mode of response. The *stress phase* was considered as the cumulative effect of responding to neutral, congruent and incongruent stimuli of the SCWT. During the *recovery phase*, the subjects were asked to relax for another 10 minutes, with their eyes closed. Continuous ECG was recorded throughout the three phases and blood pressure was measured at the end of each phase. The short-term ECG recordings of 5 minutes from each phase of the experiment was considered for HRV analysis (3). The ECG signals were ensured free of ectopic, missing data and interference noise. The ECG data was plotted as a tachogram based on the time period between two consecutive R waves (in Milliseconds). The SDNN and RMSSD were calculated from the tachogram. Using squared modulation of a Linear Fast fourier transformation, the Spectral Power density was obtained. The Total Power (<= 0.40 Hz), Low Frequency Power (0.04-0.15 Hz) and High Frequency Power(0.15-0.40 Hz) were derived and their normalized units were calculated. A measure of the Sympathovagal Balance, LF/HF ratio was also calculated (3). The HRV analysis was carried out using Finland v1.1 Software.

Statistical analysis

SPSS version 11.5 software for windows and Microsoft excel were used for statistical analysis. All values were expressed as Mean+SD. One-way analysis of variance (ANOVA) followed by post-hoc Tukey test was used in analyzing the data for the SCWT before, during and after test in both groups. Unpaired Student's t-test was used to find out the level of significance between the two groups. $P<0.05$ was considered statistically significant.

Results

In the present study, there was no significant difference with respect to age and basic parameters between the two groups. The mean BMI was found to be within the normal range in both the groups but on statistical analysis height was significantly greater

(P<0.001) in male compared to female (Table I).

HRV parameters

Table II shows comparison of HRV indices among male and female subjects. We found that there is no statistically significant difference in mean R-R interval, mean HR, SDNN and RMSSD among the two groups before the test. There was no difference between the genders during and after SCWT in mean RR, NN50, pNN50 and RMSSD. (Table III, Table IV) ANOVA test followed by post-hoc Tukey test among 3 phases revealed no significant changes in NN50, pNN50, RMSSD and mean HR (Table V). However, in the post stress phase, mean HR was found to be significantly greater in males when compared to females (Table IV).

TABLE I : Comparison of age, body weight, body mass index (BMI), mean heart rate, systolic blood pressure (SBP) and diastolic blood pressure (DBP) mean arterial pressure (MAP) of the subjects of different groups. Data presented are Mean±SD. The data analyzed using students unpaired t-test to compare the mean values between Genders (male and female).

Parameter	*Male n=25*	*Female n=25*	*P value*
Age	19.52±0.71	19.80±0.64	0.152
Ht (cm)	173.44±5.10	164.00±5.37	0.001
Wt Kg	66.72±7.98	60.24±5.94	0.102
BMI	22.13±2.00	22.39±1.90	0.648
Mean RR	770.89±113.86	743.08±102.68	0.369
Mean HR	80.20±13.21	82.62±10.88	0.484
SBP(mmHg)	115.68±7.73	114.00±5.06	0.278
DBP	80.08±6.79	77.60±6.97	0.209
MAP	91.94±5.76	89.73±5.08	0.156

Significant if P<0.05*.

TABLE II : Comparison of HRV and BP indices of the subjects of different groups (Male and Female) at rest.

Parameter	*Male n=25*	*Female n=25*	*P value*
Mean RR	770.89±113.86	743.08±102.68	0.369
Mean HR	80.20±13.21	82.62±10.88	0.484
SDNN	59.27±22.06	53.98±26.10	0.433
TP	3640.72±2517.84	3239.36±3062.29	0.615
LF ms^2	1257.28±955.55	856.72±882.14	0.130
HF ms^2	1042.28±745.61	1373.56±1597.75	0.354
LF nu	54.19±14.89	44.37±17.84	0.040
HF nu	43.63±15.59	55.49±17.83	0.016
LF/HF	1.58±1.09	1.12±1.23	0.177
RMSSD	47.44±20.93	49.34±33.48	0.811
NN50	49.34±33.48	96.36±56.94	0.921
pNN50	26.26±17.45	26.54±22.87	0.962
SBP	115.68±7.73	114.00±5.06	0.278
DBP	80.08±6.79	77.60±6.97	0.209
MAP	91.94±5.76	89.73±5.08	0.156

Values are expressed as Mean±SD; Mean RR: mean RR interval, Mean HR (bpm) : mean heart rate in beats per minute, SDNN: standard deviations of averages of normal to normal (N-N) intervals, TP: total power in absolute values (ms^2), LF: low frequency and HF: high frequency in absolute values (ms^2) LF, HF in normalized units (nu), RMSSD: root mean square of differences of successive N-N intervals, pNN50 percentage of N-N intervals, SBP: Systolic blood pressure, DBP: Diastolic blood pressure. MAP - Mean arterial pressure.

TABLE III : Gender wise BP and HRV parameters during Stroop color and word test.

Parameter	*Male n=25*	*Female n=25*	*P value*
Mean RR	746.90±117.82	713.50±94.94	0.275
Mean HR	86.14±10.76	83.14±14.73	0.416
SDNN	58.73±26.96	60.38±24.17	0.821
TP	2936.36±1989.33	2799.16±2212.71	0.819
LF ms^2	1160.48±750.11	748.20±745.63	0.051*
HF ms^2	952.08±801.65	976.12±943.82	0.923
LF nu	56.00±10.54	47.72±15.02	0.029*
HF nu	43.59±10.28	51.79±14.98	0.029*
LF/HF	1.55±0.56	1.17±0.60	0.025*
RMSSD	49.51±25.48	49.74±31.749	0.978
NN50	91.76±70.48	97.12±57.66	0.770
pNN50	25.61±16.80	23.32±19.20	0.655
SBP	120.04±5.71	113.36±5.25	0.022*
DBP	81.76±7.07	77.36±7.76	0.042*
MAP	93.52±5.96	89.36±6.06	0.018*

Values are expressed as Mean±SD; Mean RR: mean RR interval, Mean HR (bpm) : mean heart rate in beats per minute, SDNN: standard deviations of averages of normal to normal (N-N) intervals, TP: total power in absolute values (ms^2), LF: low frequency and HF: high frequency in absolute values (ms^2) LF, HF in normalized units (nu), RMSSD: root mean square of differences of successive N-N intervals, pNN50 percentage of N-N intervals, SBP: Systolic blood pressure, DBP: Diastolic blood pressure. MAP - Mean arterial pressure.

TABLE IV : Gender wise BP and HRV parameters after Stroop word and color test.

Parameter	*Male n=25*	*Female n=25*	*P value*
Mean RR	734.56±98.44	769.84±108.15	0.234
Mean HR	88.16±12.32	79.50±10.60	0.010**
SDNN	53.73±24.43	61.62±22.87	0.344
TP	3054.88±2996.24	3910.04±2809.22	0.304
LF ms^2	1685.68±1303.9	1000.28±1094.69	0.051*
HF ms^2	992.88±1102.16	1027.56±919.39	0. 904
LF nu	60.94±17.12	50.53±14.36	0.040*
HF nu	43.86±16.96	50.22±14.43	0.234
LF/HF	1.99±1.22	1.30±1.20	0.052*
RMSSD	45.43±21.25	44.47±27.39	0.978
NN50	83.52±72.04	91.60±54.86	0.770
pNN50	24.81±16.38	22.22±20.20	0.655
SBP	120.04±5.71	113.36±5.25	0.001***
DBP	81.76±7.07	77.36±7.76	0.52
MAP	93.52±5.96	89.36±6.06	0.508

Values are expressed as Mean±SD; Mean RR: mean RR interval, Mean HR (bpm) : mean heart rate in beats per minute, SDNN: standard deviations of averages of normal to normal (N-N) intervals, TP: total power in absolute values (ms^2), LF: low frequency and HF: high frequency in absolute values (ms^2) LF, HF in normalized units (nu), RMSSD: root mean square of differences of successive N-N intervals, pNN50 percentage of N-N intervals, SBP: Systolic blood pressure, DBP: Diastolic blood pressure. MAP - Mean arterial pressure. Values are significant; *(P<0.05), **(P<0.01), ***(P<0.001).

Frequency domain indices

In our study, Total Power and LF ms² showed no significant difference among two groups (Table II) at rest. In males there was a statistically significant reduction HF nu (P<0.029), statistically significant increase in LFms², LF nu (P<0.035, P<0.029) and LF-HF ratio (P<0.025) during the SCWT test (Table III). There was a significant increase in LF ms², LF nu and LF-HF ratio (P<0.051, P<0.045, P<0.052) among the males even in the recovery phase (After test) (Table IV). ANOVA test followed by post-hoc Tukey test among three phases revealed statistically significant increase in LF ms², LF nu and LF/HF ratio (P<0.026, P<0.035, P<0.04) in males and a significant decrease in HF nu (P<0.035) in males compared to females (Table V).

Blood pressure

There was no statistically significant difference in SBP, DBP and MAP at rest (Table I). However SBP, DBP and MAP (P<0.022, P<0.042, P<0.018) during the test were significantly more in males compared to females (Table III). Immediately after the test only the increase in SBP in males was statistically significant (P<0.001) (Table IV). ANOVA test followed by post-hoc Tukey test among 3 phases showed an increase in SBP and MAP in males (P<0.000, P<0.014) which were statistically significant.

Discussion

In our study, we found increased sympathetic activity in males compared to females. Also, significantly higher systolic blood pressure was found in males during the test. Earlier studies have shown that there appears to be a correlation between stress in males, the HPAA axis and the ANS control of their cardiac activity (1, 2). Male hormones play a role in decreasing vagal and increasing sympathetic activity by enhancing the cholinergic muscarinic activity at central and peripheral levels (4). It has been postulated that both physiological and psychological stress contributes to the change in blood pressure rise during SCWT (3, 4). Studies of Autonomic functions in the different phases of SCWT have reported significantly higher SBP and increased sympathetic activity in males compared to females without significant differences in parasympathetic activity (19). One study has reported that, in males, the central stress response shows changes in SBP and DBP that were significantly higher. This strongly suggests that there is activation of the sympathetic adrenal medullary axis by mental stress (20, 21). Other studies have also shown higher heart and respiration rates during mental and physical stress than at rest (22). In our study, a significant difference was observed between the two groups with respect to their overall HRV status. It was found that males had significantly reduced heart rate variability throughout the test in the form of decreased vagal

TABLE V : Comparison of BP and HRV indices before, during and after the SCWT values are expressed as Mean±SD; statistical analysis was done by one-way ANOVA test followed by post-hoc Tukey test among 3 phases (Before, During, after SCWT).

Parameter	*Before test*		*During test*		*After test*		*P value*
	Male	*Female*	*Male*	*Female*	*Male*	*Female*	
Mean RR	770.89±113.86	743.08±102.68	746.90±117.82	713.50±94.94	746.90±117.82	713.50±94.94	0.366
Mean HR	80.20±13.21	82.62±10.88	83.14±14.73	86.14±10.76	88.16±12.32	79.50±10.60	0.366
SDNN	59.27±22.06	53.98±26.10	60.38±24.17	58.73±26.96	61.62±22.87	53.73±24.43	0.805
TP	3640.72±2517.84	3239.36±3062.29	2936.36±1989.33	2799.16±2212.71	3910.04±2809.22	3054.88±2809.22	0.602
LF ms²	1257.28±955.55	856.72±882.14	1160.48±750.11	769.20±778.63	1685.68±1303.9	1000.28±294.69	0.026*
HF ms²	1042.28±745.61	1373.56±1597.75	952.08±801.65	976.12±943.82	1027.56±919.39	992.88±1102.16	0.738
LF nu	54.19±14.89	44.37±17.84	56.00±10.54	47.72±15.02	55.94±17.12	50.53±14.36	0.035*
HF nu	43.63±15.59	55.49±17.83	43.59±10.28	51.79±14.98	60.94±17.12	49.22±14.43	0.035*
LF/HF	1.58±1.09	1.12±1.23	1.55±0.56	1.17±1.03	1.99±1.22	1.30±1.20	0.045*
SBP	115.68±7.73	114.00±6.97	120.04±5.71	113.36±5.25	120.04±5.71	113.36±5.25	0.000***
DBP	80.08±6.79	77.60±6.97	81.76±7.07	77.36±7.76	81.76±7.07	77.36±7.76	0.057
MAP	91.94±5.76	89.73±5.08	93.52±5.96	89.36±6.06	93.52±5.96	89.36±6.06	0.014*
RMSSD	47.44±20.93	49.34±33.48	49.51±25.48	49.74±31.74	45.43±21.25	44.47±27.39	0.972
NN50	96.36±56.94	98.32±79.65	97.12±57.66	91.76±70.48	91.60±54.86	83.52±72.04	0.972

and increased sympathetic activity. This was reflected by lower total power, HF nu and increased mean heart rate in males. This is in accordance with a previous study by Hoshikawa et al (21). In contrast, other studies have found no differences in HRV between two groups (28) during SCWT.

Moreover LFnu, which reflects the modulation of sympathetic nervous activity and LF-HF ratio a marker of sympathovagal balance (SVB) (30), was found to be more in males. Lesser values of this ratio in females indicate increased parasympathetic and higher values in males indicate sympathetic dominance (3, 23). Other studies have also shown that psychological stress is associated with shifting of cardiac autonomic activity towards sympathetic over activity (23).

Blood pressure changes show a greater increase in systolic pressure than in diastolic pressure. This may be due to the changes in the heart rate and the fact that the diastolic pressure is mainly due to peripheral resistance, which is unlikely to be altered given the young age of these subjects.

Limitations

Our study used a small sample size in both groups. HRV was recorded for a very short duration. A 24 hours Holter recording for HRV would be a better choice to understand the cardiovascular autonomic activity. The stress provided was not subjectively quantified so as to correlate with HRV findings. Furthermore, the study can be expanded by correlating cardiac autonomic activity with plasma levels of cortisol, epinephrine and norepinepherine.

Conclusion

The findings of our study do substantiate that there is difference among the genders to SCWT Cardiovascular autonomic response is more towards sympathetic activation in males than females, even with a stress of short duration, such as the SCWT. A study of the cardiovascular autonomic response to long term stress would provide a greater insight into the patterns of sympathetic activation among the genders.

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