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STRESS RESPONSE;

PSYCHOLOGICALLY STRESSED AND CONTROL SUBJECTS; COMPARISON OF AUTONOMIC AND NEURO ENDOCRINE RESPONSE

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ABSTRACT… Introduction: Psychological stress activate two axes: Hypothalamic- Pituitary-Adrenal axis and Sympathoadrenal axis leading to production of cortisol and catecholamines. Autonomic disturbances in the body can be evaluated by estimating heart rate variability. **Study Design:** Cross sectional study. **Setting:** Islamic International Medical College. **Period:** June 2014 to December 2014. **Materials and Methods:** Subjects were labeled as stress and control on basis of DASS questionnaire proforma. Morning Cortisol level of all the subjects was measured by quantitative ELISA method. Heart rate variability recording of all the subjects was done. **Results:** Low frequency in absolute and normalized unit and low to high frequency ratio was significantly higher in stressed group, compared to control ($p \leq .05$, $p \leq .001$, $pp \leq .001$ respectively). High frequency in normalized was significantly lower in stressed subjects, compared to control ($p \leq .001$). Cortisol level was significantly higher in the stressed group in comparison with control ($p \leq .05$). **Conclusion:** Stress can lead to increase morning cortisol level and can cause autonomic disturbances which can be evaluated by measuring heart rate variability.

Key words: Stress, Low Frequency, High Frequency, Hypothalamic- Pituitary- Adrenal Axis, Sympathoadrenal Axis.

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INTRODUCTION

Stress is a precise sequence of distressing psychological and physiological responses that occur when an external stimulus challenges individual's aim.[1] Too much stress together with psychological factors can be damaging and can disrupt the ability to perform normal tasks. It can cause poor quality of life, low self esteem and affects the ability to cope with daily life problems.[2]

The two fundamental axes activated by stress are; "Hypothalamic- Pituitary- Adrenal axis (HPA)" and Sympathoadrenal (SMA) axis.[3] HPA axis activation results in activation of paraventricular neurons (PVN) of hypothalamus to release corticotrophin releasing hormone (CRH) into hypothalamic pituitary portal system. CRH travels to pituitary to stimulate the release of "adrenocorticotropic hormone (ACTH)" from the anterior pituitary. ACTH stimulates the adrenal gland to release cortisol.[4] Cortisol regulates HPA axis activation through negative feed back mechanism by inhibiting the release of CRH and ACTH from the hypothalamus and pituitary, but repeated and prolong activation of HPA axis due to stress disables cortisol of its ability of negative feedback regulation creating a state of persistent hypercortisolemia.[5]

"Autonomic nervous system (ANS)" consists of sympathetic and parasympathetic systems (SNS and PNS), both acting in balance with each other. ANS along with HPA axis plays a main role in physiological responses to stress. Sympathetic activation in stress increases heart and decreases HRV.[6] "Heart rate variability (HRV)", a noninvasive quantitative tool to evaluate cardiac autonomic function and is a predictors of mortality and morbidity.[7] It is analyzed by time and frequency domain methods.[5] "Frequency domain method" is frequently used for short term recording of HRV and are based on spectral analysis of HRV.[8] Three frequency components are usually identified by power spectral analysis, the "high frequency

(HF)" component of "HRV", spanning 0.15–0.4 Hz, is due to heart rate variation produced by respiration and primarily mediated by vagal outflow, "low frequency (LF)" component of HRV", defined as 0.05–0.15 Hz, is postulated to be influenced by sympathetic and parasympathetic system and "very low frequency (VLF) <0.04 Hz" is believed to indicate sympathetic control of heart but exact physiological significance of VLF is still not known. LF and HF are reported in normalized units to avoid skewness of distribution. LF/HF ratio shows balance between sympathetic and parasympathetic nervous systems. [9]

Psychological stress can cause development of various diseases by disturbing the autonomic balance. It can result in increase cortisol production by activating HPA axis. The aim of the study was to access the autonomic and neuroendocrine disturbances produced by chronic stress by measuring heart rate variability and morning cortisol.

METHODOLOGY

This crossectional study was conducted in physiology lab/ multidisciplinary research lab at Islamic International Medical college, Riphah University from June 2014 to December 2014 after approval from research ethical committee IIMC. Eighty healthy subjects, from both genders, ranging in age from 20- 38 years were included in study. All the subjects were healthy and free of any disease. They were grouped as stressed and control after filling DASS questionnaire profoma.[10] After taking written informed consent, the subjects were asked to report to physiology lab in morning between 7.30 to 8.30 am. Blood samples was taken for measuring morning cortisol level. Cortisol level was estimated using quantitative ELISA kit, DRG Cortisol ELISA, lot no; 43Ko84, GmbH, Germany. Weight of the subjects was recorded, subjects were made to relax for 5 minutes and their blood pressure was measured using sphygmomanometer. For recording HRV subjects were called in morning in physiology lab between 8-10.00 am. Their recording of HRV was undertaken from ten minutes ECG in sitting position using AD Instrument power lab model Yam 4/25T.

Ten minutes ECG was taken to analyze HRV, according to the standard guidelines, published by "Task Force of European Society of Cardiology and the North American Society of Pacing Electrophysiology".[11] HRV was recorded in quiet environment at ambient temperature. ECG of the subjects was recorded in a sitting position by connecting MLA 250 shielded lead wires to Bio AMP cable which was plugged in power lab. Positive electrode was connected with left wrist and negative to right wrist and ground to right leg. HRV recorded by analyzing ECG. Data in power lab was analyzed using software Labchart 7 Pro. Frequency domain was accessed using fast Fourier transform to determine "LF and HF and LF/HF ratio".

Data was analyzed using Statistical software SPSS 21 (Statistical packages for social sciences) Mean ± SD of the variables was calculated. The normality of each quantitative variable was checked through Shapiro Wilk's test. All HRV indices and cortisol were log transformed so as to avoid skewness of distribution and normality checked again. Independent sample t –test was used to compare HRV indices and cortisol among stress and control group.

RESULTS

Descriptive characteristics of stressed and control group are give in Table-I. When frequency domain indices of heart rate variability were compared in stressed and control, it was found that low frequency in absolute and normalized unit and low to high frequency ratio was significantly higher in stressed group, compared to control ($p \leq .05$, $p \leq .001$, p p $\leq .001$ respectively). High frequency in normalized was significantly lower in stressed subjects, compared to control ($p \leq .001$). No statistically significant difference was found in stressed and control group regarding high frequency in absolute units (Table-II). Cortisol level was significantly higher in the stressed group in comparison with control ($p \leq .05$) as shown in Table-II.

Characteristics	Stressed group n=40 $\bar{x}$ ±SD	Control group n= 40 $\bar{x}$ ± SD
Age (years)	36±2	33±4
Weight (kg)	72.5 ± 13	69.60 ± 12
BMI (kg/m$^{2)}$	26.07 ± 2.3	25.6 ± 2.3
Heart rate /min	79.31 ±8.93	81.30 ± 8.56

Table-I. Descriptive characteristics of stressed and control subjects

Parameters	Stressed n=40 $\bar{x}$ ± SD	Controls n= 40 $\bar{x}$ ± SD	t value	p value
LFms^2 Ref range: 1175 ms^2	677.27± 46.97	556.35± 610.04	-2.055	.04*
HFms^2 Ref range : 975 ms^2	293.28± 178.44	326.60±169.46	1.039	.302
LFnu Ref range: 56	62.71± 12.60	49.21± 12.08	-4.894	.000*
HFnu Ref range: 29	28.07 ± 11.15	40.29± 10.87	-4.656	.000*
LF/HF Ref range: 1.5-2	3.01±2.55	1.39± .904	-5.129	.000*
Cortisol Ref range; 50-230 ng/dl	122.97±68.74	98.49±41.39	-1.930	.05*

Table-II. Comparison of Heart rate variability parameters and cortisol level between stressed and control group
n= sample size
SD = standard deviation

DISCUSSION

Stress is a major risk factor for the development and progression of metabolic syndrome through various mechanisms and has been recognized as a major health problem. It can affect the cardiovascular and biochemical parameters leading to disturbance in normal physiology. Evidence based reviews have discussed the mechanisms through which stress leads to various disturbances in human physiology.

Present study observed the effects of psychological stress on HRV and cortisol. Different studies have shown increased low frequency in psychologically stressed subjects. Minakuchi et al., (2013) hypothesized that in response to stress there is increased sympathetic activation which leads to decrease HF and increase LF/HF ratio and LF in response to mental stress and it was confirmed by the results of that study.[12] Our results were in line with the findings of that study showing that sympathetic indices of HRV are increased in psychologically stressed subjects.

High frequency is an index of parasympathetic control and in current study HF was reduced in stressed subjects due to decrease parasympathetic dominance. A study conducted by Collins et al.,(2005) reported decreased HF in subjects with high job strain and low control due to decrease parasympathetic activation and increase sympathetic activation.[13] Current study reported similar findings as HF was decreased in stressed subjects showing decrease parasympathetic control. Hernandez-Gaytan et al., (2012) proposed that stress is associated with vagal withdrawl and confirmed his hypothesis by reporting low HF in doctors complaining of strain at work[14] and our results were in accordance with the findings of that study. Results of current study were consistent with the findings of Eller's et al., (2011) who reported decrease in HF component of HRV in teachers and engineers due to vagal withdrawl.[15] Takada et al., (2010) conducted a

study in Japanese worker suffering from job stress and he proved that HF was considerably lower in stressed group and when this group was given treatment, their HF increased which showed that stress was associated with vagal withdrawl.[16] Kemp et al., (2012) also showed decrease in HF in depressed subjects due to vagal withdrawl.[17] Decrease in HF and increase in low frequency in the current study proves that stress is associated with parasympathetic withdrawal as shown by decrease in HF in stressed subjects and sympathetic dominance depicted by increased LF and LF/HF ratio in stressed subjects. This imbalance has been postulated to be key mechanism for various diseases.[18]

Psychological stress results in increase in activation of HPA axis leading to increase in serum cortisol concentration. Increased in the cortisol has been reported to cause development of various metabolic diseases.[19] In this study stressed group was compared to their controls and it was found that stressed subjects had significantly higher cortisol level. Evidence based studies reported in literatures have shown an increase in cortisol level during stress due to increase activation of HPA axis. Eller et al.,(2006) reported higher salivary cortisol due to over activation of HPA axis in women with higher degree of time pressure and in men who are overcommitted at work.[20] Our findings were in line with the results of Eller et al. Significantly increased morning cortisol level in subjects with general life stress was reported in a study by Chida et al., (2009).[21] Maina et al., (2009) reported significant positive association of high psychological stress and increased morning cortisol due to excessive HPA axis activity.[22] Vreeburg et al., (2012) reported increase in salivary cortisol in a person with anxiety disorder[23] which is supported by the findings of the current study and proves that stress leads to increase in cortisol level. Results of present study were in line with the findings of above mentioned studies which proved that psychological stress increases HPA activation leading to increase in cortisol production.

CONCLUSION

Psychological stress can lead to disturbance in autonomic nervous system which can be quantified by measuring heart rate variability along with disturbed neuroendocrine response as evaluated by increased morning cortisol level.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Dr. Ghazala Jawwad	Conception and design, Data collection, Analysis and interpretation of data and drafting of research article.	
2	Dr. Humaira Fayyaz Khan	Facilitate Data collection, Analysis and critically revising the draft work.	
3	Dr. Amanat Ali	Facilitate the statistical analysis of the data.