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The Psychophysiological Responses of the Chronic Ischemic Stroke Patients to the Acute Stress were Changed

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HIGHLIGHTS

- The physiological response of ischemic stroke to acute stress (TSST) was assessed
- Patients had higher baseline cortisol level and anxiety score than health subjects
- The level of cortisol did not increase in the patients after TSST
- The stress increased the relative low frequency of HRV in both groups
- Alpha 1 of DFA of HRV of patients was significantly more than health subjects

Abstract: Mood disorder is one of the complications of stroke. The inability to cope with stress is also a prognosis of depression and anxiety. The aim of this study is to assess the response of stress system in the post stroke patients. Twelve healthy controls (HC) and twelve post-stroke patients after filling in the State-Trait Anxiety Inventory completed the Trier Social Stress Test (TSST) which induces acute stress. Salivary samples were collected to determine salivary cortisol levels and ECG record were taken in four times (before, right after stress, after two recoveries: 20 and 40 minutes after stress). ECG was also recorded during TSST and then the linear and non-linear features of Heart Rate Variability (HRV) were analyzed. The results showed that trait anxiety score and baseline salivary cortisol level were higher in post stroke than HC group (P-value <0.05). The increase of cortisol level after stress was only observed in HC that returned to baseline after the second recovery time. The stress increased the relative low frequency of HRV in both groups, however it was significantly lower in the stroke than HC group (P-value < 0.005). There was also a significant difference between alpha 1 DFA measures in stroke group and HC group (P-value <0.05). It is concluded that the impairment of the hormonal axis of stress system in the post-stroke patients that until now was not reported.

Keywords: ischemic stroke; acute social stress; cortisol; heart rate variation.

INTRODUCTION

All over the world, stroke is the second mortality cause so that 10 percent of 55 million deaths are due to the stroke[1, 2], although it is the third mortality cause in the US [3] . From 1990 to 2010, the age-standardized incidence of stroke increased by 12% in low-income and middle-income countries [4]. In Iran, the annual stroke incidence ranged from 23 to 103 per 100,000 population [5] while another study showed that the mortality rate among stroke patients is 12.1 % [6]. As stroke is becoming an increasing health problem, its complications must be getting more attention[7, 8]. The most debilitating complications of stroke are psychiatric complications[9]. Depression, anxiety and adjustment disorder are common mood disorders after stroke[10, 11].

In the general population, it is highly accepted that mood disorders is closely correlated with chronic stress [12-14] and adults with mood disorder report more physiological responses to the stressful life events[12]. Physiologically, stress stimulates amygdala-prefrontal axis in the brain and, finally, following the excitation of Paraventricular Nucleus of Hypothalamus, Hypothalamic Pituitary Adrenocortical Axis (HPA) as well as Sympathetic Adrenal Medullary axis (SAM) will be activated [15, 16]. The activation of HPA axis and SAM system is an adaptive process that enables the human body to maintain physiological stability in response to stress [17]. In addition, the activation of HPA axis eventually causes cortisol release and SAM system activation causes the increase of heart rate and blood pressure. Therefore, Heart Rate Variability (HRV) indices can be used to evaluate the autonomic nervous system activation [18].

Given the fact that ischemic stroke, as a huge source of stress, results in some changes in the autonomic nervous system, measured by HRV [19], and also it affects HPA axis for the reasons specific to stroke [20-22], we hypothesized that the physiologic responses to the psychosocial stress, like cortisol release and heart rate variability, might be different between the stroke patients and healthy individuals. Besides that, these altered physiological responses to stress in ischemic stroke patients may be one of the major etiologies for post-stroke mood disorder.

The aim of this study was to compare the cortisol level and heart rate variability facing acute stress, induced through Trier Social Stress Test (TSST), and also to compare physiologic biomarkers of chronic stress between patients with chronic stroke and healthy controls that until now was not studied.

MATERIAL AND METHODS

Participants

Twenty-two male individuals entered the study (12 healthy controls and 12 post- stroke patients). The inclusion criteria were age, 18 up to 70 years old, ischemic stroke that happened > 1 year before the study and is approved by computerized tomography (CT) scan or Magnetic Resonance Imaging (MRI)., Modified Ranking Score <=3 (in post- stroke patients), no history of other systemic diseases like diabetes, cardiovascular disease, autoimmune diseases and psychiatric diseases, no regular neurophysiologic medication uptake, no smoking at least one day before the study and no heavy exercise at least one month before the study. The health control (HC) group was matched in terms of age, gender and body mass index with patients group. All participants completed and signed the informed consent before their participation in the study. The number of clinical trial assignment is IRCT20171128037666N1.

Procedure

At the arrival, the participants were given a demographic questionnaire and State-Trait Anxiety Inventory. After filling in these questionnaires, the individuals were asked to wash their mouth and after a minute give a salivary sample. After that, a blood sample was taken. Then, they rested for 30 minutes. As the level of cortisol and heart rate variability are dependent on circadian rhythm, all the subjects were tested at the same time (between 10:00 AM to 3:00 PM that the changes of cortisol is minimum) and the same place. Salivary samples and electrocardiography (ECG) record were taken in four times (before TSST, right after TSST, after two recoveries: 20 and 40 minutes after TSST). ECG was also recorded during TSST. TSST protocol started with a 5-minute speech preparation before coming to the test room and proceeded with a 3-minute speech performance (an explanation of the job and family status) against two examiners and then completed by a 12-minute math portion. In the math portion, individuals were asked to sequentially subtract the number 13 from 1022 to the end. If the participant made a mistake, the examiners would notice them with the following sentence: "That is incorrect, please start over from 1022". The subjects stayed in standing position during the test [23]. The psychological report of anxiety by emotional visual analogue scale (EVAS) from most calm to most anxious was also taken at 4 times of the test (rest, after stress, after first and second recovery times).

Salivary cortisol measurement

The participants were asked to have enough sleep last night, not eat anything one hour before test and not have clear stress in the time of test. If they did not adhere these conditions, the test was done in the next visit. The saliva samples were obtained minimally (0.5 mL) and then transferred to a refrigerator kept at the temperature of -80 Celsius degree. The human saliva cortisol enzyme immunoassay (EIA) kit from ZellBio Company made in German was used with the procedure been done based on kit's instruction. The negative curve of TDS was determined and then the level of cortisol according to OD adjusted on the curve was assessed in each sample.

Heart rate recording

The heart rate was recorded with ECG instrument designed by Biomed Company from Iran. One electrode was attached to the left midclavicular line above the heart position, and the second electrode was attached to the left sternal border below the heart position. The third electrode was attached to the right lower quadrant of the abdomen. The subjects stayed in sitting position without deep breathing or speaking during the test. Data was saved for 2 minutes in each section (except during TSST, which lasted for 15 minutes) and relayed to an analog-to-digital converter in the sampling rate of 256 Hz. The HRV was analyzed with MATLAB software, Heart Rate Variability analysis codes. The linear features extracted from RR series in the time domain were mean and SD of RR. In the frequency domain, High

Frequency (HF) power (0.15- 0.5 Hz), Low Frequency (LF) power (0.05−0.15 Hz), very LF power (0-0.04 Hz) and finally the ratio of LF/HF components were analyzed [24]. The non-linear features extracted in time domain were SD1 and SD2 of Poincaré Plot[25], the alpha 1 of Detrended Fluctuation Analysis (DFA)[26], sample entropy and, in the frequency domain, spectral entropy (SpeEn)[27].

Statistics

The t-test was used to compare age, BMI, plasma factors, the score of spilberger's questioners, cortisol and heart rate between two groups. The chi-squared test was also used to compare the prevalence of education level between groups. To compare the cortisol level and HRV features between the sections of the study and two groups, two-way mixed model ANOVA with following related tests were used. The p-value less than 0.05 was considered as significance.

RESULTS

Twelve male post-stroke patients and twelve male healthy controls recruited in this study (mean age 56.5 in stroke group and 51.6 in healthy individuals, P-value< 0.05). Table 1 shows the comparison of demographic characteristics and biomarkers between stroke group and healthy control group. There was no significant difference in BMI and education between two groups, however trait anxiety score and baseline salivary cortisol level were significantly higher in stroke group (P-value< 0.05). Moreover, we analyzed some biomarkers (the plasma malonaldehyde as a stress oxidative enzyme and plasma glutathione and catalase as the antioxidant enzymes) as the secondary findings but there was no significant difference in biomarker levels between two groups. The result of psychological report of anxiety by emotional visual analogue scale (EVAS) showed that anxiety increased after stress in both groups and returned to baseline after recovery (F=176.15, P-value <0.00001). The level of EVAS was higher in the stroke than HC group (f=4.39, P-value< 0.05) (figure 1).

Table 1: the demographic and biochemical markers of two groups and result of comparisons between them.

	Healthy controls G.	**Ischemic Stroke G.**	**P-value**
Age(years)	51.6±3	56.5±6	0.052
BMI(weigh/Heigth^2)	26±4	25±4	0.48
Education(%Diploma and under Diploma)	76.9	92.3	0.29
Employed (%)	63.6	58.3	0.56
Modified Rankin Scale for Neurologic Disability(% slight/moderate)	-	75/25	-
Trait anxiety score	41.38±5.6	46.38±5.2	0.027
State anxiety score	36.69±6.5	36.69±6.7	1
Side of Stroke (% Both/Right/Left)	-	46.2/30.8/23.1	
Plasma malonaldehyde (µmol/L)	0.0061±0.0005	0.0064±0.0005	0.19
Plasma Glutathione(mM)	3.48±0.4	2.96±1.2	0.19
Plasma Catalase (mM)	3.59±2.4	4.87±2.2	0.2
Salivary cortisol (ng/ml)	19.2±13	43.5±28	**0.012**
Heart Rate(beats/minute)	84.52±8	85.2±11.5	0.89

Data is mean±SD, significant difference is bold data

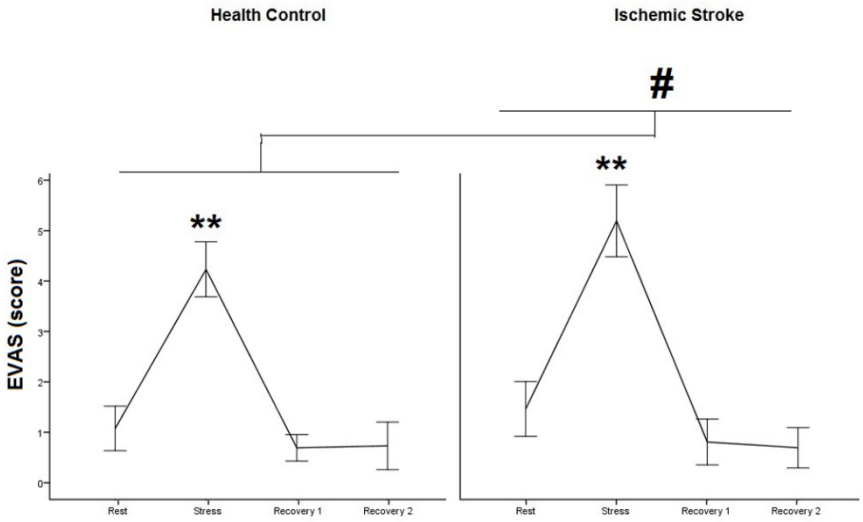

Figure 1: the plots show the mean and 95% CI of EVAS in the four times of test. **: p-value < 0.000 between stress and rest time per groups. #: p-value< 0.05 between two groups.

Salivary cortisol

Figure 2 shows the salivary cortisol level at four times of assessment in both groups. The results of Two-way mixed model ANOVA test showed that the interaction effect of time and group was significant (F 5.09, P-value < 0.008). It means that healthy individual's salivary cortisol levels significantly increased after the induced stress and it remained high during the first 20-minutes recovery time, but it began to decrease to the baseline levels during the second recovery time. While, unlike healthy controls, the salivary cortisol level of stroke patients decreased after stress, but this change was not statistically significant (figure 2).

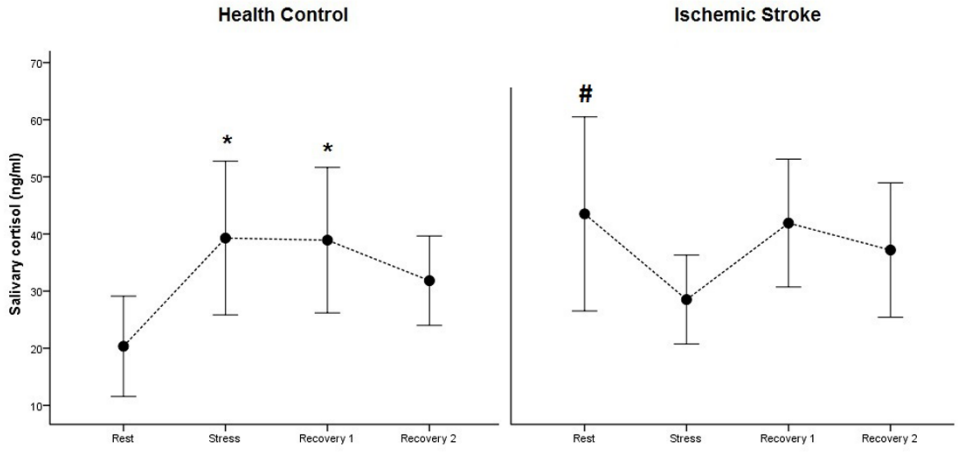

Figure 2: the plots show the mean and 95% CI of salivary cortisol at four times of measurement and in two groups (Healthy control and ischemic stroke groups). *: significant difference between the rest and other conditions. #: significant difference between two groups in the rest.

The features of HRV

As shown in Figure 3, the number of heart beats per minute increased after the induced stress. As a result, the R-R intervals reduced in both groups, but these changes were significantly more in stroke patients (F=21.97, P-value <0.001). After stress and during recovery time, the R-R intervals restored to the baseline level.

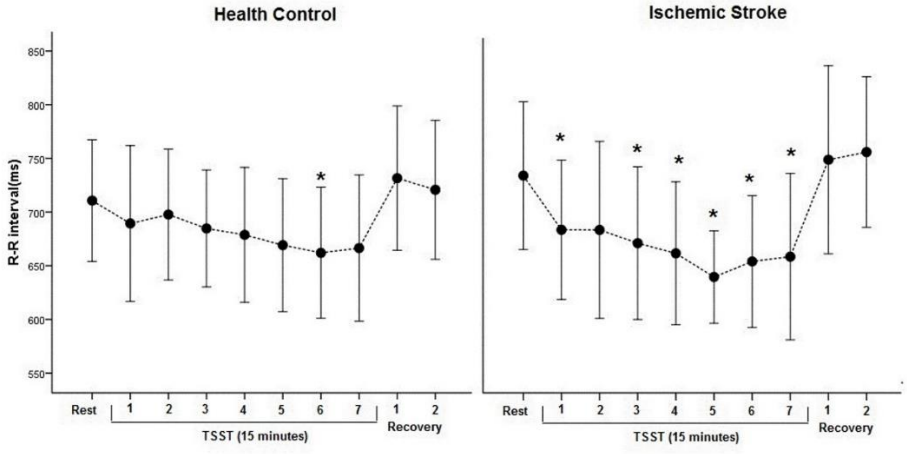

Figure 3: the plots show the mean and 95% CI of R-R interval of HRV in the repeated measurements in the rest, stress and recovery time. *: significant difference between the rest and other conditions.

Besides that, two-way mixed model ANOVA showed no significant difference in SD of R-R interval of HRV in the repeated measurements at the rest, stress and recovery times between two groups (figure 4). As shown in figure 4, the diversity of SD of R-R interval was more between the stroke patients than the healthy controls, and the same finding was

obtained for the SD1 of Poincare plot of HRV in the repeated measurements in the rest, stress and recovery time (figure 5).

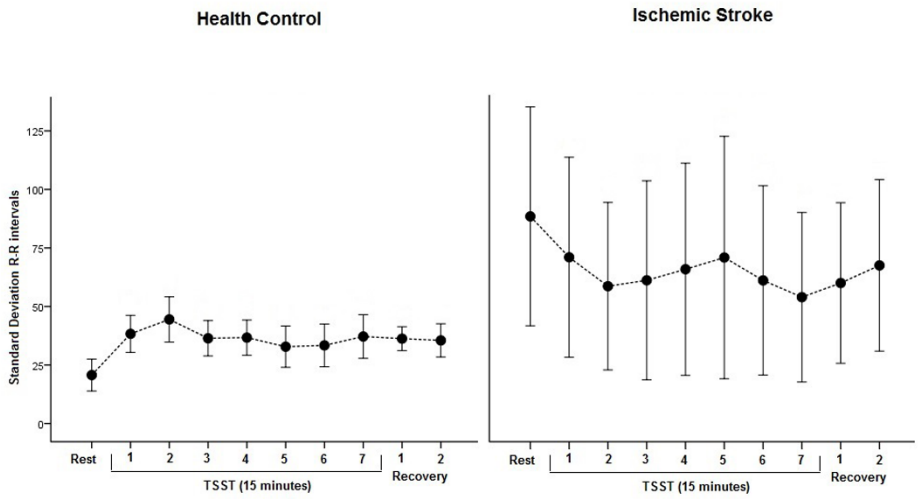

Figure 4: the plots show the mean and 95% CI of SD of R-R interval of HRV in the repeated measurements in the rest, stress and recovery condition.

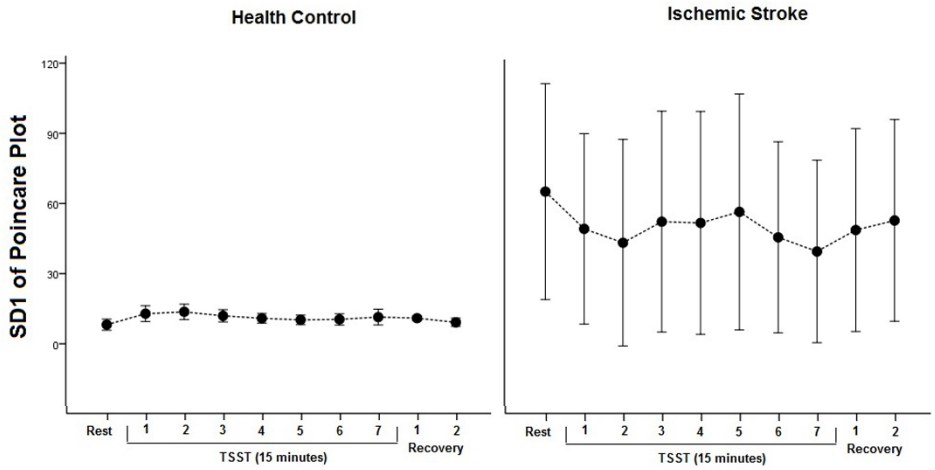

Figure 5: the plots show the mean and 95% CI of SD1 of Poincare plot of HRV in the repeated measurements in the rest, stress and recovery condition.

In the analysis of the relative power frequency of HRV, two-way mixed model ANOVA showed that the interaction effect of time and group was significant (F=2.04, P-value < 0.05). as shown in figure 6, the relative low frequency (LF) of HRV increased in both groups during the stress phase and came back to the baseline after recovery time, but, totally, the relative low frequency of HRV was significantly lower in the stroke group (P-value < 0.005). The changes of the relative low frequency of HRV during the stress phase and recovery time were more significant in the healthy individuals so that there was a statistically significant difference in the relative low frequency of HRV in healthy individuals between the second recovery time and the second two minutes of stress phase (P-value <0.05). The difference in the relative low frequency of HRV between the fifth two minutes of stress phase and the rest time in the stroke group was also significant (P-value <0.05).

The relative high frequency (HF) of HRV decreased significantly in both groups sequentially (F=3.17, P-value < 0.05) and there was no significant difference between stroke patients and healthy controls (figure 7). There were no significant differences in the LF/HF and relative power of VLF between two groups and between different phases of the protocol.

As shown in figure 8, there was no difference in the alpha 1 DFA between rest time, stress phase and recovery time, but there was a significant difference in alpha 1 DFA measures between stroke group and healthy controls group (P-value <0.05). Also, there was no difference in spectral and sample entropy measures between different times and different groups.

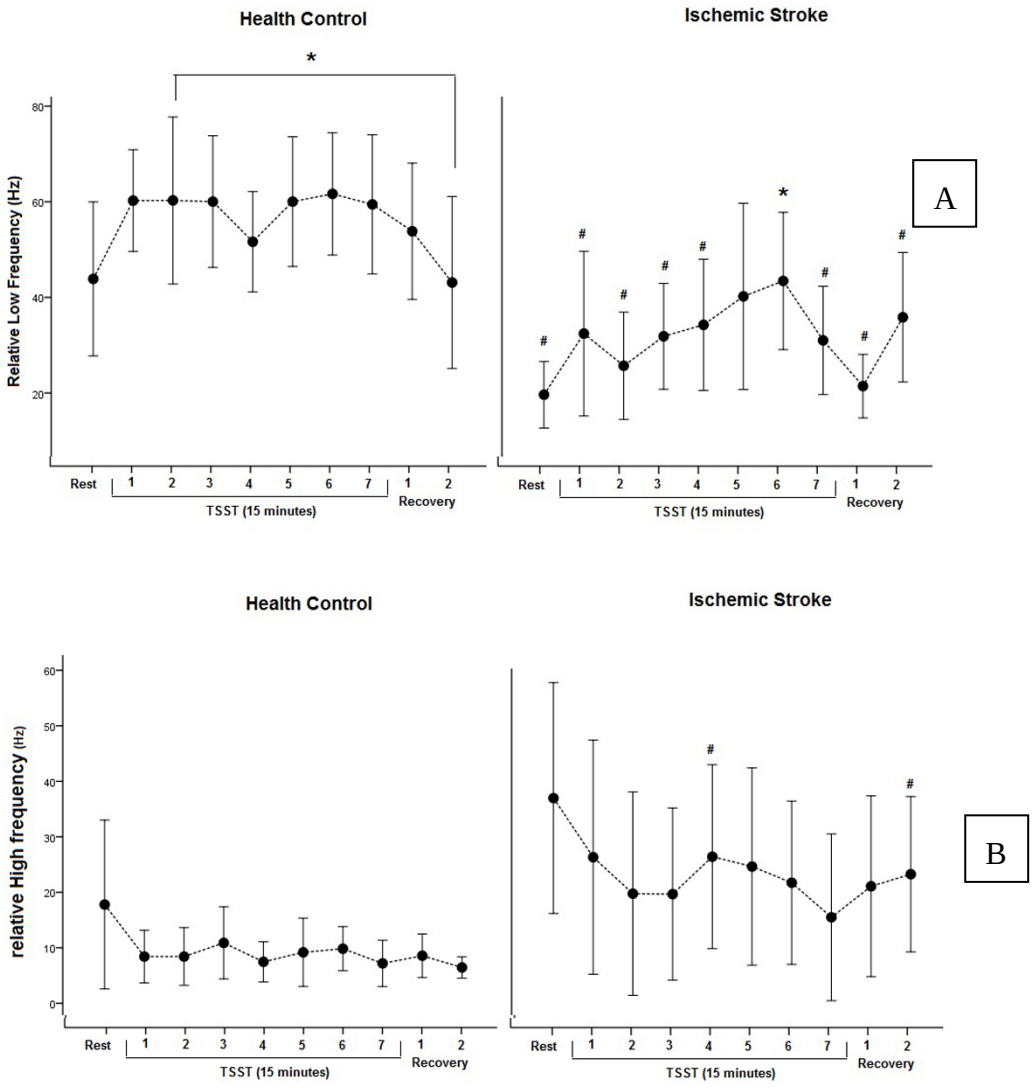

Figure 6: the plots show the mean and 95% CI of relative low frequency of HRV (A) and high frequency of HRV (B) in the repeated measurements in the rest, stress and recovery condition. *: significant difference between conditions within group. #: significant difference between two groups in the same condition.

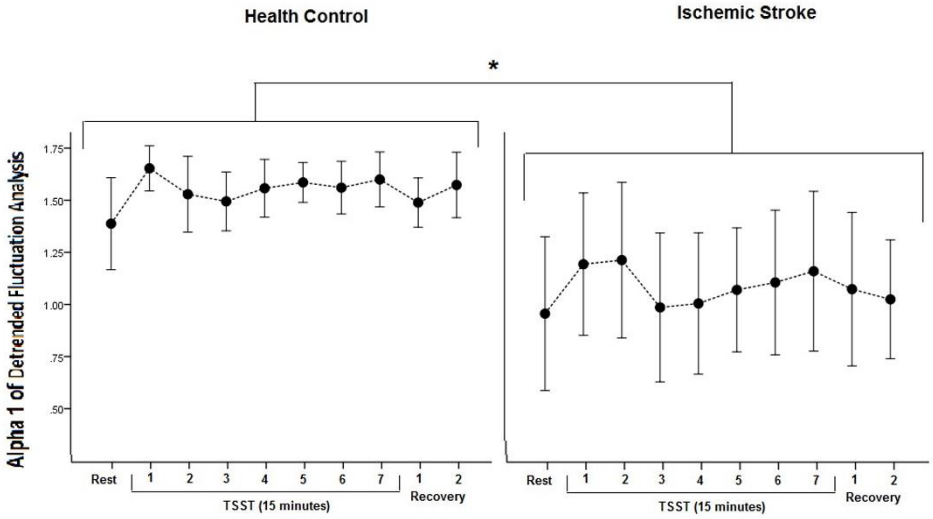

Figure 7: the plots show the mean and 95% CI of alpha 1 of DFA in the repeated measurements in the rest, stress and recovery condition. *: significant difference between within group

DISCUSSION

In the present study, in order to assess the stress system response in the post-stroke patients, TSST protocol was used. In both post-stroke and HC groups, the anxiety significantly increased after TSST based on EVAS score. Then, it was returned to baseline after recovery. The baseline comparison showed that stroke patients significantly had higher trait anxiety score. A recent systematic review involving 39 cohorts consist of 4,706 stroke patients showed that 24% of them had anxiety symptoms and 18% had an anxiety disorder in the first 5 years after stroke[28]. On the other hand the anxiety is a complication due to chronic stress[16]. Then there is a close chain of stroke, stressful events and mood disorder might be suggested.

HPA axis assessment

The change of salivary cortisol is the marker of HPA activity [16]. Basically, the resting salivary cortisol levels and anxiety score were higher in post-stroke patients than in healthy controls. After inducing stress, salivary cortisol levels raised in healthy controls and maintained after the first twenty minutes of recovery, while salivary cortisol tended to decrease non-significantly after stress in post-stroke patients. Some recent studies showed that cortisol levels in post-stroke patients are higher than healthy individuals [22, 29]. Our finding supports the previous studies. Also, we found that anxiety level at the resting time and EVAS score after acute stress were higher in post-stroke patients than in healthy controls, which is probably due to the underlying ischemic stroke mechanism [30]. In fact, the higher level of anxiety at the resting time and higher EVAS score after stress could be a precursor for the higher level of salivary cortisol. Previous studies showed that the increase of cortisol after stress has negative correlation with baseline cortisol [31, 32]. It is noticeable that, in our study, we also pointed out that the patients with chronic stroke responded to the acute stress with salivary cortisol decrease. This is also consistent with a previous study which showed that the cortisol level decreases after an acute stress in chronic stroke patients [33]. Then this study confirmed that chronic stroke patients exposed to chronic stress that showed no increase of cortisol after acute stress. Chronic stress increases the anxiety behavior and depression and decreases the production of brain-derived neurotrophic factor (BDNF) even in the offspring[34]. It is suggested to measure the level of BDNF that is a pathophysiological predictor for depression[35].

SAM axis assessment

The HRV features are the markers of SAM activity [36]. The results showed that the heart rate increased after acute stress and returned to the resting level during recovery time in both groups. The decrease of R-R internal as a marker of heart rate during the stress was more significant in the post-stroke group. After acute stress, the LF power of HRV significantly elevated in both groups and then returned to the resting level after recovery which means that the sympathetic tone increased in both groups after acute stress. Basically, the LF power was significantly lower and HF power was significantly higher in the post-stroke group than healthy controls which means that the parasympathetic tone increased in post-stroke patients. This finding is in line with the previous findings confirming that in post-stroke patients parasympathetic overactivity occurrs [37]. Another important finding of our study was that SD of RR intervals, SD1 and SD2 of Poincaré Plot values, which both measure HRV volatilities, were very discrepant between post-stroke patients after stress, and there was less discrepancy in these values between healthy groups. This finding might be due to the different impacts of different locations of ischemic stroke on HRV [38]. Besides that, the non- linear features of HRV, like SD of Poincare, alpha 1 DFA, sample entropy in time domain and spectral entropy in the frequency domain (SpeEn), were not significantly different after and before acute stress in both post-stroke patients and healthy controls, despite of previous studies which showed that acute stress causes a decrease in the heart rate variability in the young voluntaries [36].This mismatch between our finding and previous studies is probably contributable to the higher average age of participants in our study than previous studies, considering these facts that the HRV decreases in old ages [39] and the brain functions and physiologic responses in older ages are diminished, so we should use modified HRV indices for different ages [40]. It is also notable that, in consistence with previous studies, we found significant lower mean values of alpha 1 of DFA in the post-stroke group at the resting time, which means that stroke causes a decrease in HRV [41, 42].

Many studies investigated cardiac complications and heart rate variability changes after stroke [42-45]. For example, in an earlier study, Fyfe-Johnson et al. found that the lower HF is correlated with the higher risk of stroke in adults [46]. In some other studies, it is shown that the patients with ischemic stroke had Autonomic Nervous System (ANS) dysfunction and decreased HRV compared to the healthy controls [19, 41], and this decreased HRV increases the stroke severity and risk of mortality [47]. As it was predictable, we found that LF of HRV significantly increased after stress and HF of HRV decreased at the same time in both groups, which can be interpreted as the increase of sympathetic tone due to stress [48].

Our study has some limitations. First of all, we only enrolled patients with chronic ischemic stroke because the patients in the acute phase of stroke cannot participate in TSST protocol. The second limitation of our study was our limited sample size. So, we recommend that the future studies evaluate the physiological responses of acute stroke patients to the acute stress in the larger sample sizes.

CONCLUSION

In conclusion, in this study, we found that the post-stroke patients had different physiologic responses to the acute stress in comparison with healthy individuals. The post-stroke patients showed the increase of SAM activity based on HRV features due to stress but not HPA activity based on cortisol level. The non-linear HRV features of patients were very different that might be due to the different impaired region of brain, and need future research. The parasympathetic tone was also higher in post-stroke patients than HC group. The baseline trait anxiety score and salivary cortisol of patients significantly higher than HC and the change of cortisol due to TSST did not happen significantly in patients. Thus, the patients had signs of chronic stress and exposed them to several impairments such as mood disorders. The measurement of BDNF protein or gene is suggested to confirm in term of molecular assessment.

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