Research Article

The Relationship between DASS-42 Questionnaire, Salivary Cortisol and Heart Rate Variability

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Running title: DASS-42 questionnaire and biological factors

Abstract

Background and Objectives: Cortisol and heart rate variability (HRV) represent the activity of physiological stress axes. The depression and anxiety stress survey (DASS-42) has been widely used to assess stress. This study examines the correlations between the DASS-42 questionnaire and stress markers, salivary cortisol levels and HRV.

Methods: A total of 195 healthy volunteers (145 males and 50 females) participated in this study. At first, the DASS-42 survey form was completed. The salivary cortisol samples were collected, and the electrocardiograms were recorded, respectively. Differences in cortisol between baseline and post-trier social stress tests were recorded as changes in cortisol after stress (CCAS). Measurements were made to establish whether the overall DASS and its

subscales (stress, anxiety, and depression) were correlated with baseline cortisol, CCAS, and HRV indices.
Results: The anxiety-DASS subscale score correlated negatively with the CCAS score in women (r = -0.429, P = 0.002). The DASS score was significantly correlated with heart rate (r = 0.25, P = 0.007) and SD2 of Poincare plot (r = -0.272, P = 0.004) in men. In contrast, women showed significant correlations between total DASS scores with very low-frequency power (r = -0.40, P = 0.005) and detrended fluctuation analysis-α1 (r = -0.30, P = 0.034). The anxiety-DASS subscale correlated with HRV in both genders.
Conclusion: The anxiety-DASS subscale represents the sympathetic-adrenal medulla activity. Clinicians can estimate the activity of this stress axis by using the anxiety subscale of DASS questionnaire. Meanwhile, gender differences should be noted when assessing stress.
Keywords: Anxiety, Salivary cortisol, Heart rate, Nonlinear analysis

1. Introduction

Most health researchers currently agree that stress is critical for human health and aging (1). Stress is associated with an increased risk of various diseases, such as cardiovascular disease, hypertension, and even infectious diseases. The measurement of stress, which lacks consistency and thoroughness, is inherently complex (2).
Stress simultaneously activates two axes: the hypothalamus-pituitary-adrenal (HPA) and the sympathetic-adrenal medulla (SAM). The activation of the HPA leads to increased cortisol in body fluid and the activation of the SAM affects heart function (1, 3)**.** The correlation between salivary cortisol and plasma cortisol was greater than 0.9 (4). Therefore, many of studies in basic and clinical sciences are using salivary cortisol as a biological marker to examine the activity of the physiological nervous system or brain stress system.
Another promising stress marker used is heart rate variability (HRV), which is the variation in the length of the heartbeat interval. Time-domain measurements of HRV include assessing the quality of the mean or standard deviation (SD) of the RR interval. Frequency-domain measurements of HRV, including the assessments of the percent of power of the high-frequency (PHF) component and the percent of power of the low-frequency (PLF) component, were calculated. In addition to the mentioned linear indicators, nonlinear features include SD1 and SD2 of the Poincaré plot and α1 of the detrended fluctuation analysis (DFA) (3, 5).
the depression, anxiety, and stress (DASS-42) questionnaire has been used to assess stress state instead the physiological responses as stress markers (cortisol and HRV). DASS-42 developed by Matthews et al., It has been shown that has high reliability and validity (Cronbach's α of 0.96 to 0.97 for DASS-Depression, 0.84 to 0.92 for DASS-Anxiety, and 0.90 to 0.95 for DASS-Stress). The questionnaire consists of 42 items divided into three sections and reports the levels of depression, anxiety, and stress (6).
Given the variety of methods employed to assess stress, it is possible to establish a link between the results of self-reports and physiological responses to stress. Physiological responses are a potential pathway linking stress to some morbidities or diseases (7). If this relationship is confirmed, a standard questionnaire could be used to estimate an individual's health status and even indicate possible future health risks, potentially saving time and money. Several studies have reported this association (1, 7). Some of these studies were conducted in children (8) or adolescents (9) with limited sample sizes, and only one study used the DASS-42 questionnaire(10). The participants in these studies were in specific physiologic states, such as after curettage or after an earthquake (9, 11).
The importance of using the DASS questionnaire as an efficient tool for stress assessment will be examined. The first hypothesis of this study is the relationship between DASS scores and physiological biomarkers of stress (salivary cortisol and heart rate variability) in healthy

subjects, as HRV and cortisol have been shown to reflect subjects' reports of stress. Secondly, HRV and cortisol may be in good agreement.

2. Materials and Methods

2.1. Study Participants

The study design is cross sectional. This study was conducted among healthy volunteers in Tehran City, Iran from March 2021 until September 2023. After surveying 500 volunteers, 195 among them were considered eligible and participated in the study and were referred to laboratory of neuroscience research center of Baqiyatallah university of medical sciences. A total of 195 individuals (145 males and 50 females) between the ages of 25 and 50 years participated in the study.

The inclusion criteria comprised physical and mental health, no smoking habits, no history of craniocervical surgery or mental illness, no history of systemic diseases (such as diabetes or cardiovascular disease) (based self-report and clinical psychologist comment), and no participation in a regular exercise program in the past month. Meanwhile, all participants had to comply with the research Ethics Code IR. BMSU. REC.1400.051 and they signed an ethics consent form approved by Baqiyatullah Medical University of medical sciences.

2.2. Study Procedure

After spending 5 min in the testing room, the participants first completed the DASS-42 questionnaire, and then 5 mL of cortisol saliva between 9-15 o'clock and 2 minutes of ECG was collected from the participants. Testing was performed while all variables at baseline were recorded. Only saliva samples were collected twice (before and after the trier social stress test [TSST]). The TSST protocol consisted of an interview and a math task, which included five minutes of speech preparation before entering the testing room, a 2-min speech performance for two managers, and an 8-min math portion. The participants were asked to successively subtract the number 13 from 1022. The examiner verbally reported the answers aloud and asked them to start in 1022. If the participant made a mistake, the examiner prompted, "You are wrong, please start over from 1022." (3).

2.3. Study Outcomes

2.3.1. Depression and Anxiety Stress-42 Questionnaire

The DASS-42 questionnaire was completed in the quiet environment of the exam room. The participants were carefully instructed to complete the questionnaire according to their living conditions. The form was divided into three sections and consisted of 14 questions reporting levels of depression, anxiety, and stress based on scores obtained on a 5-point scale (10, 12).

2.3.2. Salivary Cortisol Level

Minimal (0.5 mL) saliva samples were collected and frozen at -80°C. After thawing, a human saliva cortisol enzyme-linked immunosorbent assay (ELISA) kit from IBL, Germany, was used. The ELISA was performed according to the kit instructions (3).

2.3.3. Electrocardiography Recording

Electrocardiography (ECG) recording was performed using equipment from Rib Intelligence Technology, Iran. Three electrodes were used in this study. The first electrodes were placed on the midline to the left of the heart. The second electrode was placed in the right lower abdomen, and the third electrode was placed on the left edge of the sternum, below the heart position. HRV can be affected by many factors, including circadian rhythms, body position, level of physical activity before testing, medication intake, speech impairment, and deep breathing. For this reason, special care was taken to ensure that all users were under the same conditions.

ECGs were recorded at the same time of day. During the study, the subjects were in a sitting position without deep breathing or talking. ECG recordings were performed for 2 min using standard methods (13, 14). Heart Rate Variability Software, developed by John T. Ramshur (2010) in MATLAB (8), was used for HRV analysis. At first, the HRV was extracted from the ECG signal by appropriate filtering, and peaks were identified during the analysis process. Since the ECG signal is bandpass filtered between 5-20 Hz, the peak R can be estimated by setting the amplitude threshold value and the minimum time interval between consecutive peaks. In the time domain, the mean and SD of the RR were extracted as linear features from the RR series. In the frequency domain, high frequency (HF) power (0.15-0.5 Hz), low frequency (LF) power (0.05-0.15 Hz), ultra-LF power (0-0.04), and the ratio of LF/HF were analyzed. (5). The nonlinear features extracted in the time domain are SD1 and SD2 from Poincaré plots and $\alpha 1$ from detrended fluctuation analysis (DFA), and in the frequency domain, spectral entropy (Spectral-ENT)(3, 15, 16).

2.4. Statistical Analysis

After the data were collected, they were analyzed using the SPSS software (version 25). Since the data were normal based on the results obtained by the Kolmogorov–Smirnov normality test, the Pearson correlation coefficient was used to determine the relationships among the variables. Curve estimation was used to determine the nonlinear correlation between cortisol and the frequency domain of HRV (percent of VLF and HF). The significance level for all the tests was $P < 0.05$.

3. Results

A total of 195 males and females participated in the study. Table 1 shows the mean SD and sex comparisons of the baseline data. There were no significant differences in the DASS depression score, DASS anxiety score, or heart rate between men and women. In contrast, the DASS-total score and DASS-stress score were significantly greater in men, and the baseline cortisol level was significantly greater in women (Table 1).

Table 1. Comparison of mean and SD of DASS scores and baseline cortisol levels between the two genders

Variables	Men	Women	*P* Value
DASS	33.37±23.02	25.46±20.97	0.02*
DASS-depression	8.90±8.62	7.86±8.09	0.44
DASS-anxiety	8.12±6.66	7.06±6.21	0.30
DASS-Stress	13.62±9.39	10.42±8.73	0.03*
Baseline-cortisol	6.74±4.02	13.60±6.61	0.00*
Heat rate	78.73±11.58	80.66±11.10	0.31

Abbreviations: DASS, depression, and anxiety stress survey. *: significant difference of the T-Test

3.1. Relationships of the Depression and Anxiety Stress Survey Total Score and Its Subscale Score with Salivary Cortisol

There was no significant relationship between the DASS total score and baseline cortisol or CCAS scores in men and women. There was no relationship between the three DASS subscales and salivary cortisol in men; however, in women, only the DASS-anxiety subscale showed a significant negative correlation with the CCAS score ($r = -0.429$, $P = 0.002$) (Table 2).

Table 2. DASS scores relationship with CCAS

Gender			DASS	DASS-Depression	DASS-Anxiety	DASS-Stress
men	Cortisol	*R* p-value	-0.040 0.636	-0.106 0.210	-0.007 0.933	-0.147 0.081
	CCAS	*R* p-value	-0.095 0.292	-0.117 0.192	-0.120 0.183	-0.129 0.151
women	Cortisol	*R* p-value	0.115 0.427	0.158 0.272	0.128 0.368	0.142 0.326
	CCAS	*R* p-value	-0.194 0.178	-.041 0.780	**-.429*** **.002**	-0.211 0.141

Abbreviations: DASS, depression, and anxiety stress survey; CCAS, changes in cortisol after stress. Significant showed as bold data. *: significant Pearson Correlation

3.2. Relationships Between the Depression and Anxiety Stress Survey-Total Score and Its Subscale Score and Heart Rate Variability

For men, the DASS total score showed a statistically significant positive correlation with heart rate ($r = 0.250$ and $P = 0.007$) and a statistically significant negative correlation with Poincare plot-SD2 ($r = -0.272$ and $P = 0.004$). The depression subscale score did not significantly correlate with HRV. The anxiety subscale showed a significant positive correlation with HR ($r = 0.282$, $P = 0.002$) and a significant negative correlation with the mean RR ($r = -0.25$, $P = 0.006$) and Pioncare plot-SD2 ($r = -0.291$, $P = 0.002$). The stress subscale showed a significant negative correlation with Pioncare plot-SD2 ($r = -0.218$, $P = 0.002$) and a positive correlation with spectral entropy ($r = 0.214$, P = 0.027) (Table 3). There was no significant correlation between DASS and its subscales with sample entropy and DFA-alpha1 of HRV.

Table 3. DASS scores relationship with HRV in men

		Mean RR	SD	HR	PVLF	PLF	PHF	LF/HF	Poincare plot -SD1	Poincare plot -SD2	SPECT -ENT
DASS	*R* p-value	-0.230 0.014	-0.058 0.537	**0.250*** **0.007**	0.106 0.261	-0.80 0.398	-0.038 0.686	-0.015 0.877	-0.065 0.490	**-0.272*** **0.004**	0.177 0.068
DASS-depression	*R* p-value	-0.118 0.210	0.028 0.768	0.143 0.128	0.035 0.711	0.083 0.384	0.036 0.704	-0.087 0.369	0.024 0.797	-0.094 0.329	0.026 0.789
DASS-anxiety	*R* p-value	**-0.255*** **0.006***	-0.110 0.244	**0.282*** **0.002**	0.106 0.262	-0.50 0.596	-0.064 0.498	0.019 0.845	-0.104 0.272	**-0.291*** **0.002**	0.130 0.183
DASS-stress	*R* p-value	-0.156 0.096	-0.065 0.493	0.167 0.076	0.048 0.615	-0.120 0.205	0.055 0.562	-0.103 0.287	-0.016 0.862	**-0.218*** **0.022**	**0.214*** **0.027**

Abbreviations: DASS, depression, and anxiety stress survey; SD, standard deviation; HR, heart rate; PVLF, very low-frequency power; PLF, low-frequency power; PHF, high-frequency power; LF/HF, Low frequency/high frequency; Spect-ENT, spectral entropy. Significant showed as bold data. *: significant Pearson Correlation

In women, the total DASS score was significantly positively correlated with the PVLF (r = 0.405, P = 0.005) and DFA-α1 (r = 0.309, P = 0.034). Depression subscale scores were significantly positively correlated with the PVLF (r = 0.422, P = 0.003) and DFA-α1 (r = 0.338, P = 0.019) and significantly negatively correlated with the PHF (r = -0.324, P = 0.003). Anxiety showed a significant positive correlation with PVLF (r = 0336, P = 0.020) and DFA-α1 (r = 0.291, P = 0.043). The stress component showed a positive correlation with only the PVLF (r = 0.390, P = 0.07) (Table 4). There was no significant correlation between DASS and its subscales with sample entropy and spectral entropy of HRV.

Table 4. DASS scores Relationship with HRV in women

		Mean	SD	HR	PVLF	PLF	PHF	LFHF	Poincare plot -SD1	Poincare plot -SD2	DFA -α1
DASS	*R* p-value	-0.105 0.483	- 0.189 0.202	0.095 0.526	**0.405*** **0.005**	0.069 0.647	-0.281 0.059	0.142 0.345	-0.284 0.053	-0.080 0.594	**0.309*** **0.034**
DASS-Depression	*R* p-value	-0.107 0.471	- 0.197 0.181	0.085 0.564	**0.422*** **0.003**	0.111 0.459	**-0.324*** **0.026***	0.202* 0.174*	-0.282 0.052	-0.107 0.469	**0.388*** **0.019**
DASS-anxiety	*R* p-value	-0.102 0.487	- 0.193 0.183	0.122 0.403	**0.366*** **0.020**	0.126 0.393	-0.281 0.053	0.120 0.417	-0.252 0.080	-0.115 0.430	**0.291*** **0.043**
DASS-stress	*R* p-value	-0.059 0.691	- 0.109 0.461	0.049 0.739	**0.390*** **0.007**	0.011 0.941	-0.230 0.120	0.115 0.441	-0.229 0.117	0.019 0.900	0.242 0.097

Abbreviations: DASS, depression, and anxiety stress survey; SD, standard deviation; HR, heart rate; PVLF, very low-frequency power; PLF, low-frequency power; PHF, high-frequency power; LF/HF, Low frequency/high frequency; Sample-ENT, sample entropy; DFA, detrended fluctuation analysis; Spect-ENT, spectral entropy. Significant showed as bold data. *: significant Pearson Correlation

3.3. Relationship Between Salivary Cortisol and Heart Rate Variability

In men, the baseline cortisol only showed a significant negative correlation with the SD of RR (r = -189, P = 0.048). In women, the baseline cortisol was significantly related to the PVLF (r = 0.309, P = 0.031) and DFA-α1 (r = 0.296, P = 0.037) (table 5). An inverted regression was observed between salivary cortisol and both the PVLF (r = 0.361, P = 0.00003) and PHF (r = 0.233, P = 0.003) in all subjects (both sexes) (Figure 1 and Figure 2).

Table 5. Relation Between Salivary Cortisol and HRV

Gender			Mean	SD RR	HR	PVLF	PLF	PHF	Sample - ENT	DFA -α1	SPECT

											-ENT
men	**Cortisol**	*R* p-value	-0.028 0.769	**-0.186*** **0.048***	0.052 0.584	0.120 0.202	-0.011 0.905	-0.113 0.234	-0.049 0.640	-0.134 0.162	0.018 0.858
women	**Cortisol**	*R* p-value	-0.097 0.502	.022 0.881	0.130 0.369	**0.309*** **0.031**	0.132 0.367	-0.280 0.051	**-0.141** **0.0412***	0.296 0.037	-0.089 0.700

SD, standard deviation; HR, heart rate; PVLF, very low-frequency power; PLF, low-frequency power; PHF, high-frequency power; LF/HF, Low frequency/high frequency; Sample-ENT, sample entropy; DFA, detrended fluctuation analysis; Spect-ENT, spectral entropy. Significant showed as bold data

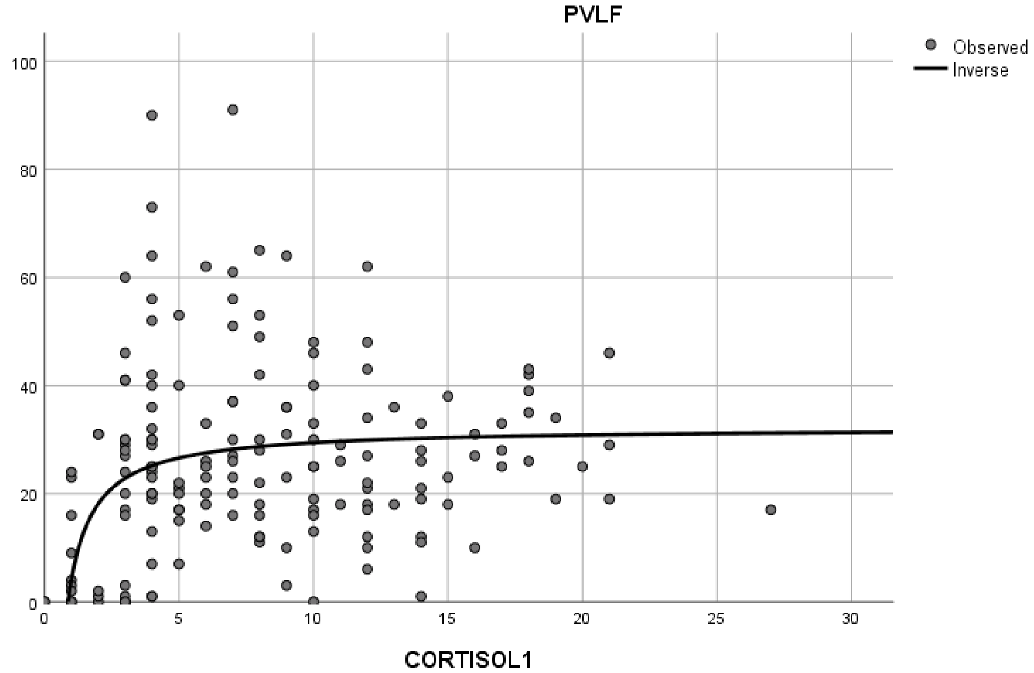

Figure 1. The Inverted Nonlinear Regression Between the Amount of Salivary Cortisol and the Very Low-Frequency Power in All Subjects (n = 195; $r = 0.361$, $P = 0.00003$).

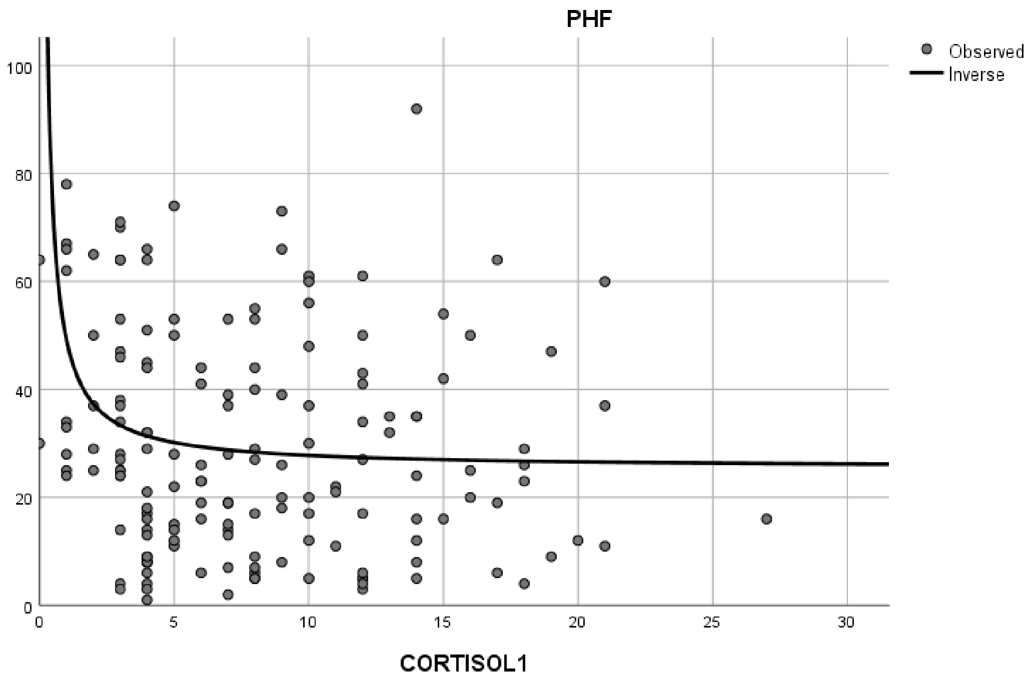

Figure 2. The Inverted Nonlinear Regression Between the Amount of Salivary Cortisol and High-Frequency Power in All Subjects (n = 195; $r = 0.233$, $P = 0.003$).

4. Discussion

This study determined the possible relationships between self-reported measures of stress, DASS-42 questionnaire and physiological biomarkers. Our results showed there was no association between total scores on the DASS or its subscale and baseline cortisol or CCAS levels. But, the DASS-anxiety subscale score was inversely related to changes in cortisol levels after stress (CCAS) in women. Consequently, two HRV characteristics (HR and Poincare plot-SD2 in men) and (PVLF and DFA-α1 in women) correlated with the total score on the DASS. In both men and women, the DASS-anxiety subscale score was correlated with the HRV score.

4.1. Comparison of the Depression and Anxiety Stress Survey-42 Score with the Baseline Cortisol Concentration and Changes in Cortisol After Stress Score

The present study analyzed baseline data from women and men and revealed that baseline cortisol levels in women were approximately twice as high as those in men. This result is consistent with previous studies showing that baseline cortisol levels of women more than men. (17, 18). Overall, women had significantly higher morning cortisol levels than men, and women under 50 years of age had higher morning and Δ-cortisol (morning and evening cortisol) levels than matched men (19). In contrast, in a study conducted among different sexes, serum cortisol levels were greater in depressed men than in depressed women (20). The results of this study differ in that the participants were healthy, and cortisol was determined in saliva rather than in serum. Salivary cortisol sampling is not stressful or inconvenient for participants and several samplings are possible.

In addition, absolute cortisol and CCAS values were not related to the total DASS or DASS subscales, except for the anxiety-DASS subscale in women, which was negatively correlated with the CCAS. This study is consistent with previous research (20, 21), in which they showed that absolute cortisol levels do not correlate with emotional self-reports of distress anxiety. In contrast, Sungako et al. reported a significant relationship between DASS scores and salivary cortisol levels in women after curettage (10). These results may be related to the characteristics of the participants examined after curettage, where hormonal fluctuations are stronger. On the other hand, some studies have suggested a circadian rhythm of cortisol that the lowest cortisol levels occur around midnight and that the peak of cortisol production occurs in the early morning. They determined the cortisol arousal response and estimated the diurnal cortisol levels by sampling several times during the day (22, 23). It was not possible to evaluate these items in this study. Meanwhile, the current results were observed in healthy young people and might be different from those in other age groups.

Our study showed that DASS scores of men were significantly greater than women. On the other hand, among employees of engineering colleges in Nigeria, no significant differences were found in DASS-42 scores (8) between males and females. In Iran, no study has compared the performance of the DASS-42 and its subset between males and females. This result can be explained given that the questionnaire is subjective, men in Iran face more work-related stresses, and in most cases, men are the only working members of the family (24).

4.2. Depression, Anxiety Stress Survey-42 Data Versus Heart Rate Variability

Theoretically, HRV may be affected by behavioral factors, such as social and conduct disorders (25). According to our results, some features of HRV are related to DASS-42.

In men, HRV characteristics, such as HR and Pioncare-SD2 showed the strongest correlation with the DASS-42 questionnaire. Nonlinear HRV characteristics, such as Pioncare-SD2, indicating the dynamic state of the heart. The main advantage of studying nonlinear characteristics is to provide more complete and natural information about biological systems [26]. No previous study using nonlinear features, such as the Poincaré-SD2 score determined their correlation with self-report questionnaire scores.

In addition to the Pioncare-SD2 and HR subscales, the DASS-anxiety subscale was correlated with the mean RR. In contrast, some studies did not observe significant changes in HRV after acute stress in men (3, 15, 16). In men, the time domain features of the HRV were correlated with those of the DASS-42.

In contrast, in women, the frequency domain of the HRV was related to the DASS-42 score and its subset. Several frequency HRV parameters have been used to test which of these parameters is most strongly associated with stress, and they represent high frequency, which can reflect vagal parasympathetic innervation. Low frequency is a dual innervation of sympathetic activity and vagal innervation (PA+SA), and the low-frequency/high-frequency ratio has been assumed to represent the sympathy-vagal balance (26). Moreover, anxiety is related to both low PA and high SA in adults and children (27). As expected, our research revealed a direct relationship between PLF and DFA-α1 in addition to an inverse relationship with PHF. This result aligns with that of Mohammadi et al., who reported changes in the frequency of HRV after stress in women(17).

The relationships between the DASS-42 and HRV showed that only two subscales, the DASS-anxiety and DASS-stress subscales, were related to HRV in men, but all three subscales of the DASS-42 were related to HRV in women. underlying mechanisms could be the sex differences that have been demonstrated in the used HRV parameters (28) in psychological functioning and development of genders since childhood (29) and in handling stressful situations (29).

For both genders, the DASS-anxiety subscale is related to HRV. The DASS-anxiety subscale questions focus more on physiological changes following stress. In this section, the state of breathing, the amount of sweating, the irregularity of heart rhythm, and the feeling of weakness are examined. It seems that this subset has more concentrated on subjective stress perception and coping with it (30). Then it maybe suggested than the DASS questionnaire evaluate the SAM axis of stress than HPA axis.

4.3. Cortisol Versus Heart Rate Variability

HRV and cortisol have been shown to reflect physiological changes after stress. We investigated their relationship. For the first time, we also consider differences between the sexes.

A low HRV (a greater sympathetic over parasympathetic dominance) is related to a greater cortisol awakening response (31). In another study, cortisol awaking response was associated with low high frequency and low frequency, but there was no cross-sectional change in low frequency/high frequency (32). Nevertheless, no significant relationship between cortisol and HRV has been reported (33, 34).

In this study, baseline cortisol showed a negative correlation with the SD of the RR in men and a positive correlation with the PVLF in women. A previous study showed that baseline cortisol has a negative correlation with the standard deviation (SD) of the RR and a positive correlation with LF without mentioning differences between men and women(17).

The relationship between cortisol and the HRV index shows the real interaction of the HPA and SAM axes in the heart. When the cortisol concentration increases to the desired level, the VLF index increases and the HF index decreases. Thus, at very low and very high suboptimal cortisol levels, the parasympathetic nervous system activity index, i.e., HF, will be above the optimal state, and the sympathetic nervous system activity index, i.e., VLP, will be below the optimal state based on cortisol levels.

Despite this relationship between HRV and cortisol, their responses to acute stress may differ. For example, it has been reported that there is a dissociation in the response of both nervous systems to repeated exposure to stress and that after several repeated exposures (habituation), the stress response of cortisol decreases while the HRV response remains high such as some disorders, multiple sclerosis, and stroke (14, 15, 35). In addition, salivary cortisol levels continued to increase under stress conditions but rapidly decreased under control conditions. Hatef et al demonstrated SDs of Poincare plots of HRV increased and the changes of salivary cortisol tend to optimal range after Muslim praying. It means the changes of HRV and cortisol are in a same way but did not have linear correlation(36, 37)

4.4. Study Limitations

One of the limitations of our study was that the number of male and female participants was not the same; the other limitation was the cortisol awakening response and diurnal decline, and their relationships with DASS and HRV were not investigated.

5. Conclusion

The present study showed that only DASS-anxiety was associated with CCA in women. The time domain of HRV was related to the DASS score in men and the frequency domain of HRV in women. This study showed that the DASS-anxiety subscale reflects SAM activity in both men and women. Therefore, clinicians can make appropriate decisions regarding this stress axis by evaluating the DASS questionnaire and, in particular, the anxiety subscale. In addition, cortisol is associated with several characteristics of HRV.

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7. Declarations

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Conflict of interest
There are no conflicts of interest.

Informed consent
Informed consent was obtained from all individual participants included in the study.

Author Contributions
Study conception and design: B.H., S.M., AS., EM; data collection: B.H., S.S., F.S., H.CH. and MZ ; analysis and interpretation of results: F.S., S.M., H.CH.,A.Sand B.H.; draft manuscript preparation: all authors. All authors reviewed the results and approved the final version of the manuscript .

Ethical approval IR. BMSU. REC.1400.051 of Baqiyatallah University of Medical Sciences, approval date: 20201-12-21

Data availability statement
The data that support the findings of this study are available upon request from the corresponding author

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