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Original Article

Serum sex hormone levels and heart rate variability as predictors for women with generalized anxiety disorder

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Abstract: Objective: To investigate the role of serum sex hormone levels (progesterone, testosterone) and heart rate variability (HRV) indicators (SDNN, LF/HF) in women with generalized anxiety disorder (GAD). Methods: A total of 155 female GAD patients admitted to Shandong Mental Health Center were selected as the research group (RG) and all received venlafaxine sustained release tablets. Another 111 female patients who underwent physical examination during the same period were selected as the control group (CG). The progesterone, testosterone, SDNN, LF/HF of the two groups were measured, and the anxiety of the two groups was evaluated by the anxiety self-rating scale (SAS) for analysis. Results: The progesterone, testosterone and standard deviation of NN intervals (SDNN) in the RG were greatly lower than those in the CG, while low-frequency and high-frequency (LF/HF) was the opposite. The area under the curve (AUC) of female GAD diagnosed by progesterone, testosterone, SDNN and LF/HF alone was 0.822, 0.810, 0.826 and 0.803 respectively, and the corresponding AUC of joint diagnosis of progesterone, testosterone combined with SDNN and LF/HF was 0.902, 0.902, 0.900 and 0.906. In the RG, the SAS score was negatively correlated with progesterone, testosterone and SDNN, while it was positively correlated with LF/HF. The post-treatment progesterone, testosterone and SDNN elevated markedly in the RG, while LF/HF was reversed. The AUC of menopause, progesterone, testosterone, SDNN and LF/HF in efficacy prediction of female GAD was 0.869, 0.766, 0.824 and 0.760, and the four were the risk factors affecting the occurrence of female GAD. Conclusion: Serum progesterone and testosterone levels, SDNN, and LF/HF can be used as predictors of diagnosis, anxiety degree, and efficacy of female GAD patients.

Keywords: Sex hormone levels, heart rate variability, female generalized anxiety disorder, prediction

Introduction

Generalized anxiety disorder (GAD) is a common mental disorder that appertains to anxiety disorder, which brings huge medical and physical burden to patients [1]. According to epidemiological data, the lifetime risk of GAD is 11.0%, while only 36.3% of GAD patients receive treatment, and the disease is more prevalent in women [2, 3]. Today, the screening tools of GAD include GAD-2 and GAD-7. However, the sensitivity and specificity of these two are not ideal, and they lack certain convenience due to the questionnaire evaluation [4]. As to the treatment, current treatment options for GAD consist of psychotherapy including mood-modulation therapy, cognitive-behavioral therapy, and medications represented by pregabalin and venlafaxine sustained-release tablets [5-8]. Studies have reported that long-term drug therapy can overcome the high recurrence rate of GAD and prevent the relapse of related symptoms in GAD patients. In addition, venlafaxine has a credible effect on treating GAD patients, which makes venlafaxine sustained release tablets the ideal research drug for GAD patients [9, 10]. Studying the diagnosis and efficacy prediction methods of female GAD patients will be conducive to improving the treatment rate and cure rate of GAD patients.

The pathological mechanism that women are more susceptible to developing GAD is potentially related to sex hormones, which play a key role in the production and maintenance of GAD, while heart rate variability (HRV) is of predictive value in the development of GAD-

related diseases [11, 12]. Sex hormones are biological modulators for the body to cope with environmental stress. Studies have shown that progesterone can reduce the risk of GAD by regulating the balance of intestinal flora. Low progesterone and testosterone levels in serum or saliva are often associated with the occurrence and progression of GAD in women [13-15]. While HRV is an indicator of abnormal heart rate changes, which can reflect the healthy state of autonomic nervous system in response to environmental changes. The R-R standard deviation of NN intervals (SDNN) presents a significant downward trend in GAD patients, while the low-frequency and high-frequency (LF/HF) ratio is opposite, which can help us screen potential GAD patients [16].

At present, there are few studies on the diagnosis and efficacy prediction of serum sex hormones and HRV in female GAD patients. Therefore, this study evaluated the diagnostic and efficacy prediction value by monitoring these two indicators, hoping to provide clinical reference value for the diagnosis and treatment of female GAD patients.

Materials and methods

General information

A total of 155 female GAD patients admitted to Shandong Mental Health Center from March 2016 to December 2018 were selected as the RG, all receiving venlafaxine sustained release tablets, aged 20-76 years, with an average age of 45.06±8.14 years. In addition, 111 female patients who underwent physical examination during the same period were selected as the CG, aged 22-75 years, with an average age of 44.74±6.82 years. This study was approved by the Medical Ethics Committee of Shandong Mental Health Center, and all the research participants and their families were informed and signed the informed consent. Inclusion criteria: Patients diagnosed as GAD [17] after the first onset without any prior treatment, who had not received any surgery in the past half a year, those without allergy to the medication used in this study, with normal cognitive function and communication. Exclusion criteria: Those who had taken any drug within the last 3 months that had a potential impact on the results of this study; those with serious organic or systemic diseases or malignant tumors; those with infectious diseases; those who had alcohol dependence in the past 3 months. The inclusion criteria were applicable to the patients in the RG, and the healthy subjects in the CG were healthy controls.

Treatment methods

All patients in the RG were treated with venlafaxine sustained-release tablets (LK0959, Roark Pharma-Tec Co., Ltd., Shenzhen, China) at 75 mg/d, followed by an increased dose of up to 225 mg at a 4-day interval, depending on patients' condition.

Outcome measures

Participants in both groups were taken 5 mL of elbow venous blood on an empty stomach at 8-9 am and placed in a vacuum vessel containing EDTA-K2 for a 10-minute centrifugation at 820 g. Next, 2 mL of the upper plasma was absorbed and transferred to the EP tube, and centrifuged at 1500× g at 4°C for 10 min to precipitate cell fragments. The supernatant was then stored in a new EP tube at -80°C for later use. The serum progesterone and testosterone of the two groups were determined by enzyme-linked immunoadsorption assay (ELISA) [18], with strict reference to the human progesterone ELISA kit and the human testosterone ELISA kit (JK-(EA)-11046, JK-(EA)10088, Jingkang Bio-engineering Co., Ltd., Shanghai, China). While SDNN and LF/HF were measured by HRV analyzer (SA-3000P, Hanfei Medical equipment Co., Ltd., Shanghai, China). What's more, self-rating anxiety scale (SAS) [19] and Hamilton anxiety scale (HAMA) [20] were adopted to evauate the anxiety of the two groups, and Hamilton depression scale (HAMD) [21] was employed to assess the depression of the two groups.

Statistical analysis

GraphPad Prism 6 (GraphPad Software, San Diego, USA) was used for the plot of the collected data in this study. The counting data were expressed by the number of cases/percentage (n/%), and the inter-group comparison was performed by the chi-square test. When the theoretical frequency in chi-square test was less than 5, the continuous correction chi-square test was applied. The measurement data were expressed as mean ± SD, and the

Table 1. Baseline data of patients in the two groups [n (%), mean ± SD]

Factors	n	CG (n = 111)	RG (n = 155)	χ^2/t	P
Menopause				10.999	<0.001
No	131	68 (56.82)	63 (54.74)		
Yes	135	43 (43.18)	92 (45.26)		
Age (years old)				0.300	1.076
<45	129	58 (55.68)	71 (51.58)		
≥45	137	53 (44.32)	84 (48.42)		
Average age (years old)	266	44.74±6.82	45.06±8.14	0.338	0.736
BMI (kg/m^2)	266	24.05±2.37	24.31±2.52	0.850	0.396
Educational level				0.749	0.862
Primary shool	70	29 (46.59)	41 (40.00)		
Junior high school	79	35 (35.23)	44 (37.89)		
Senior high school or technical secondary school	64	24 (35.23)	40 (37.89)		
Junior college or above	53	23 (39.77)	30 (28.42)		
Marital status				0.767	0.381
Married	121	54 (60.23)	67 (71.58)		
Single or widowed	145	57 (60.23)	88 (71.58)		
History of hypertension				1.466	0.226
No	106	49 (68.18)	57 (65.26)		
Yes	160	62 (31.82)	98 (34.74)		
History of diabetes				0.885	0.347
No	118	53 (65.91)	65 (73.68)		
Yes	148	58 (34.09)	90 (26.32)		
Drinking history				0.021	0.886
No	114	47 (36.36)	67 (40.00)		
Yes	152	64 (63.64)	88 (60.00)		
Smoking history				0.142	0.707
No	121	52 (39.77)	69 (33.68)		
Yes	145	59 (60.23)	86 (66.32)		
Residence				0.048	0.827
Rural	101	43 (26.14)	58 (22.11)		
Urban	165	68 (73.86)	97 (77.89)		
HAMA (points)	266	10.15±3.13	30.85±7.18	28.486	<0.001
HAMD (points)	266	8.24±1.58	15.62±2.77	25.271	<0.001
SAS (points)	266	39.84±6.54	54.87±8.36	15.792	<0.001

inter-group comparison was conducted by the independent sample t-test, while the intra-group comparison before and after treatment was realized by the paired t-test. Pearson correlation coefficient was used to evaluate the correlation between SAS score and progesterone, testosterone, SDNN, LF/HF. The multivariate logistic regression analysis was carried out by means of SPSS22.0 (Easy Biotechnology Co., Ltd., Beijing, China) to analyze the risk factors affecting GAD in women. P<0.05 indicated a statistically significant difference.

Results

Baseline data

Except a significant difference in menopause (P<0.05), the other baseline data such as age, mean age, body mass index (BMI), educational level, marital status, history of hypertension, history of diabetes, drinking history, smoking history, residence, HAMA, HAMD and SAS did not show any marked differences between the two groups (P>0.05) (**Table 1**).

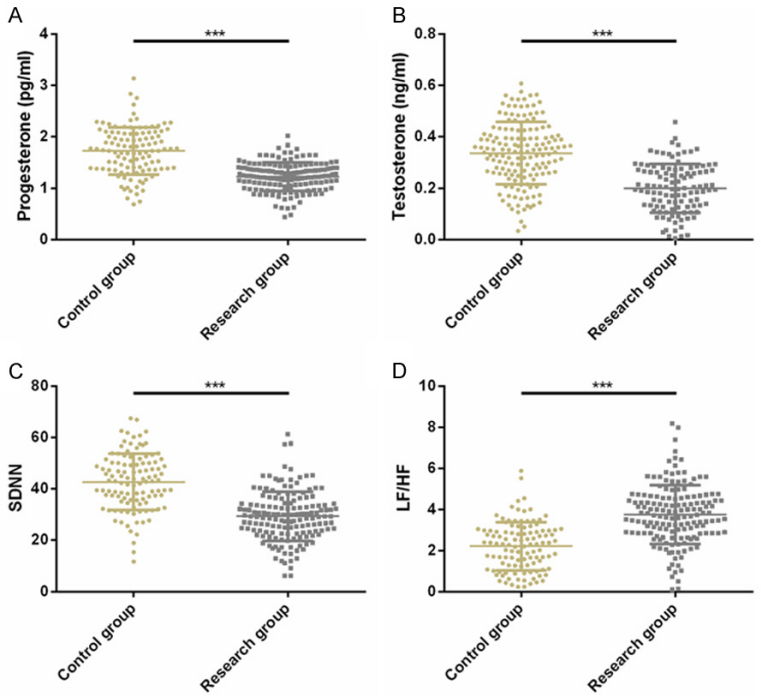

Figure 1. Progesterone, testosterone, SDNN and LF/HF levels in the two groups. A. The progesterone in the RG was dramatically lower than that in the CG. B. The testosterone was notably lower in the RG than in the CG. C. The SDNN in the RG was greatly lower than that in the CG. D. The LF/HF in the RG was markedly higher than that in the CG. Note: *** indicated P<0.001.

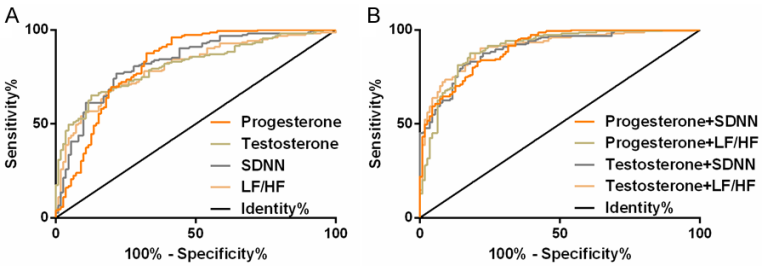

Figure 2. Diagnostic value of progesterone, testosterone, SDNN and LF/HF. A. The AUC of progesterone, testosterone, SDNN, and LF/HF in diagnosing female GAD was 0.811, 0.822, 0.833, and 0.844, respectively. B. The AUC of progesterone, testosterone combined with SDNN and LF/HF in diagnosing female GAD was 0.911, 0.922, 0.933 and 0.944, respectively.

Progesterone, testosterone, SDNN and LF/HF levels in the two groups

The levels of progesterone, testosterone, SDNN and LF/HF in the RG and CG were 1.23±0.27 pg/ml and 1.72±0.47 pg/ml, 0.20±0.09 ng/ml and 0.34±0.11 ng/ml, 28.97±10.25 and 42.68±11.02, 3.78±1.51 and 2.21±1.16. The above data indicated that, compared with the CG, the progesterone, testosterone and SDNN in the RG were notably lower, while LF/HF was higher, and the differences were statistically significant (P<0.05) (**Figure 1**).

Diagnostic value of progesterone, testosterone, SDNN and LF/HF

We drew the ROC curve of progesterone, testosterone, SDNN and LF/HF in the diagnosis of female GAD. The results showed that the AUC of progesterone, testosterone, SDNN and LF/HF for female GAD diagnosis was 0.822, 0.810, 0.826 and 0.803 respectively, and the AUC of progesterone, testosterone combined with SDNN and LF/HF in diagnosing female GAD were 0.902, 0.902, 0.900 and 0.906, respectively (**Figure 2**; **Table 2**).

Relationship between progesterone, testosterone, SDNN, LF/HF and SAS scores in the RG before treatment

There was a significant negative correlation between progesterone, testosterone, SDNN and SAS scores in the RG before treatment (r = -0.652, P<0.001; r = -0.571, P<0.001; r = -0.600, P<0.001), while LF/HF and SAS scores were significantly positively correlated (r = 0.573, P<0.001) (**Figure 3**).

Progesterone, testosterone, SDNN, and LF/HF levels in the RG after treatment

After treatment, the progesterone, testosterone and SDNN boosted remarkably in the RG, while the LF/HF declined notably, with statistically significant differences (P<0.05). The AUC of the progesterone, testosterone, SDNN, and LF/HF for efficacy prediction was 0.869, 0.766,

Table 2. ROC parameters of diagnosing female GAD patients with progesterone, testosterone, SDNN and LF/HF

Indexes	AUC	95% CI	S.E	Cut-off	Sensitivity (%)	Specificity (%)
Progesterone	0.822	0.767-0.876	0.028	1.51	87.74	67.57
Testosterone	0.810	0.760-0.861	0.026	0.30	65.16	87.39
SDNN	0.826	0.775-0.877	0.026	34.51	76.77	78.38
LF/HF	0.803	0.751-0.856	0.027	3.19	67.10	81.98
Progesterone + SDNN	0.902	0.866-0.937	0.018	0.62	83.23	79.28
Progesterone + LF/HF	0.902	0.863-0.941	0.020	0.58	87.74	81.98
Testosterone + SDNN	0.900	0.864-0.936	0.018	0.61	81.29	84.68
Testosterone + LF/HF	0.906	0.870-0.942	0.018	0.48	90.32	78.38

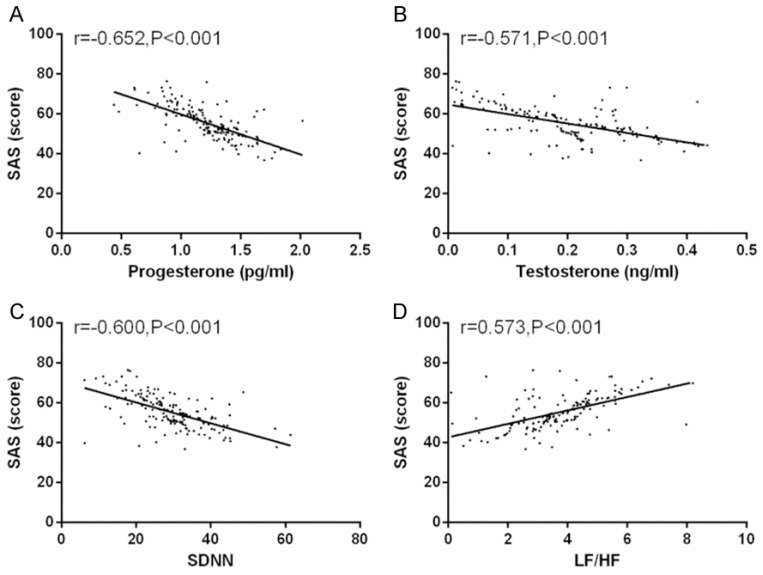

Figure 3. Relationship between progesterone, testosterone, SDNN, LF/HF and SAS scores in the RG before treatment. A. There was a significant negative correlation between serum progesterone and SAS scores in the RG (r = -0.652, P<0.001). B. There was a marked negative correlation between serum testosterone and SAS scores in the RG (r = -0.571, P<0.001). C. There was a notable negative correlation between SDNN and SAS scores in the RG (r = -0.600, P<0.001). D. There was a obvious positive correlation between LF/HF and SAS scores in the RG (r = 0.573, P<0.001).

0.824 and 0.760, respectively (**Figure 4**; **Table 3**).

Risk factors affecting GAD in women

We included progesterone, testosterone, SDNN and LF/HF in the analysis, and listed them as dependent variables for evaluation. We then took whether or not female GAD occurred as the dependent variable, and included the factors with differences (menopause) in the Logistic regression model for multivariate analysis. The results showed that menopause (P = 0.029), progesterone (P = 0.020), testosterone (P = 0.012), SDNN (P = 0.004), and LF/HF (P = 0.001) were indepedent risk factors for GAD in healthy women (**Tables 4**, **5**).

Discussion

Generalized anxiety disorder (GAD) is a kind of mental illness caused by environmental or extreme stress, and its clinical symptoms mainly manifest in anxiety, worry and nervousness, which inflicts a great impact on the life and psychology of patients. Its etiology is related to environmental factors and heredity [22, 23]. GAD is more prone to affect perinatal women, and its incidence after delivery even exceeds that of depression, which has a potential negative impact on the physical and mental health of most postnatal women and their newborns [24]. Therefore, it is of great significance to study the diagnosis and efficacy prediction of female GAD patients to improve their quality of life as well as physical and mental health.

There are extensive studies probing into the diagnosis, efficacy prediction and other related clinical applications of patients with GAD. For example, in the study by Findikli et al. [25], high serum levels of G-protein coupled estrogen receptor 1 were significantly associated with anxiety levels in GAD patients, and could

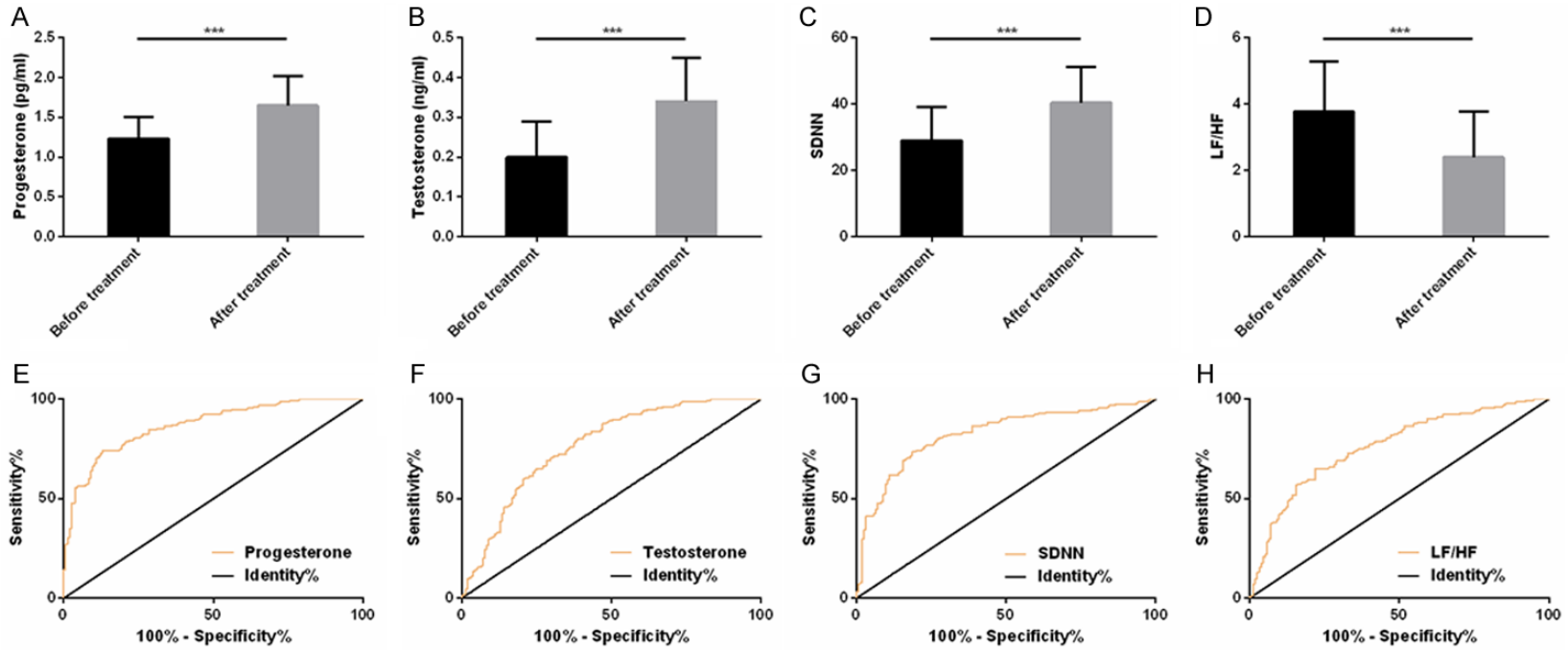

Figure 4. The ROC curve of progesterone, testosterone, SDNN, LF/HF levels and the efficacy of these four in the RG after treatment. A. Serum progesterone increased markedly in the RG after treatment. B. Serum testosterone elevated remarkably in the RG after treatment. C. SDNN boosted significantly in the RG after treatment. D. LF/HF reduced dramatically in the RG after treatment. E. The AUC of serum progesterone to predict the efficacy of patients was 0.869. F. The AUC of serum testosterone to predict the efficacy of patients was 0.766. G. The AUC of SDNN to predict the efficacy of patients was 0.824. H. The AUC of LF/HF to predict the efficacy of patients was 0.760. Note: *** indicated P<0.001.

Table 3. ROC parameters of progesterone, testosterone, SDNN and LF/HF in diagnosing female GAD patients

Indexes	AUC	95% CI	S.E	Cut-off	Sensitivity (%)	Specificity (%)
Progesterone	0.869	0.830-0.908	0.020	1.49	74.19	87.10
Testosterone	0.766	0.713-0.819	0.027	0.32	82.58	58.71
SDNN	0.824	0.777-0.872	0.024	37.20	73.55	81.29
LF/HF	0.760	0.707-0.814	0.027	2.82	65.16	78.06

Table 4. Logistic multivariate regression analysis assignments

Factors	Variables	Assignments
Menopause	X1	No = 0, yes = 1
Progesterone	X2	Continuous variable
Testosterone	X3	Continuous variable
SDNN	X4	Continuous variable
LF/HF	X5	Continuous variable

Table 5. Multivariate analysis of GAD in women

Factors	β	S.E	Wald	P	OR	95% CI
Menopause	1.110	0.487	4.713	0.029	2.989	1.121-7.975
Progesterone	1.033	0.472	5.291	0.020	2.834	1.158-6.730
Testosterone	3.762	0.169	7.216	0.012	2.104	1.047-4.136
SDNN	1.284	0.596	4.425	0.004	2.323	1.134-5.015
LF/HF	3.845	0.318	8.793	0.001	4.856	2.245-9.987

be used to predict gender-independent GAD. In another report by Khandaker et al. [26], serum C-reactive protein, a marker of inflammation, was closely related to the clinical symptoms of GAD patients, and its abnormally high levels in GAD patients may have the function of indicating the anxiety level of patients. And as reported by Shen et al. [27], neurotrophic factors (BDNF, GDNF) had a certain predictive effect on the remission of GAD patients after treatment. In this study, we selected progesterone, testosterone, indicators of sex hormones, and SDNN, LF/HF, indexes of HRV, to analyze the diagnosis and efficacy prediction of GAD patients. The results showed that the RG had notably lower levels of progesterone, testosterone, SDNN and markedly higher LF/HF level, suggesting that the four may be involved in the occurrence and progression of GAD. Further analysis of the diagnostic value of the four revealed that the AUC of progesterone, testosterone, SDNN, LF/HF for single diagnosis of female GAD was 0.822, 0.810, 0.826, and 0.803, respectively, while the AUC of joint diagnosis of progesterone, testosterone combined with SDNN, LF/HF was 0.902, 0.902, 0.900, 0.906, indicating that the combination of sex hormone and HRV indexes could significantly improve the diagnostic value of female GAD. It was worth mentioning that the testosterone combined with LF/HF enjoyed the highest diagnostic value.

Furthermore, we analyzed the correlation between progesterone, testosterone, SDNN, LF/HF and SAS scores. Among them, SAS is a scoring tool that can be used to measure anxiety-related physical and emotional symptoms in GAD patients, and its score is in direct proportion to the severity of anxiety [28]. Our correlation results indicated that progesterone, testosterone, SDNN were markedly negatively correlated with SAS scores in the RG before treatment, while LF/HF and SAS scores were remarkably positively correlated, suggesting that progesterone, testosterone, SDNN, LF/HF may be used to indicate the degree of anxiety of female GAD patients. What's more, we explored the association between the four and female GAD patients before and after treatment, as well as their predictive value. It turned out that the progesterone, testosterone and SDNN were all significantly increased, while LF/HF was greatly decreased in the RG after treatment, and the AUC of the four was 0.869, 0.766, 0.824 and 0.760, respectively, suggesting that the four possessed varying degrees of higher predictive value for the efficacy of female patients with GAD, among which progesterone enjoyed the highest predictive value for the efficacy. We finally analyzed the risk factors affecting the occurrence of GAD in women, and the results displayed that healthy women with menopause, low levels of progesterone, testosterone, and SDNN, and high level of LF/HF had an increased risk of GAD. Gillin et al. [29] reported

that sleep disorder was also a risk factor for GAD. In the research by Bentley [30], GAD was a risk factor for suicidal thoughts and behaviors, suggesting that effective treatment of GAD patients was beneficial to reduce the emergence of suicidal thoughts and behaviors in humans.

To sum up, serum progesterone and testosterone levels, SDNN and LF/HF are of high value for the diagnosis of female GAD patients, as well as for the prediction of anxiety level and treatment efficacy of patients. In particular, testosterone combined with LF/HF can be used as a potential biomarker for female GAD patients. However, there is still room for improvement in this study. First, we can increase the included indicators of sex hormones and HRV and conduct a more detailed comparative analysis to obtain independent or combined indicators with higher diagnostic performance. Second, we can supplement the toxic and side effects of this treatment to analyze the safety of venlafaxine sustained-release tablets for female GAD patients. Last but not the least, we can further discuss the risk factors that affect the ineffectiveness of venlafaxine sustained-release tablets in female GAD patients.

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Disclosure of conflict of interest

None.

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