



Altered Neurotransmitter Levels with Post-Traumatic Stress Disorder

Post Travmatik Stres Bozukluğunda Nörotransmitter Düzeylerindeki Değişim

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ABSTRACT

AIM: The aim of this study was to observe the changes in the monoamine neurotransmitter levels of patients with post-traumatic stress disorder (PTSD) and explore whether the neurotransmitter level is correlated with the severity of PTSD.

MATERIAL and METHODS: A total of 15 PTSD patients without any physical injuries were enrolled into the study. Another 15 cases of pre-restoration patients were recruited as controls who experienced traumatic events but did not experience PTSD or had a Posttraumatic stress disorder Check List-Civilian version (PCL-C) score lower than 12 points. The levels of plasma monoamine neurotransmitters, including norepinephrine, 5-hydroxytryptamine, and dopamine, were tested using enzyme-linked immunosorbent assays.

RESULTS: No significant differences were found between the monoamine neurotransmitter levels of the PTSD and the control groups ($p > 0.05$). A correlation study confirmed that the monoamine neurotransmitter levels and the PCL-C scores of the PTSD group were not significantly correlated ($p > 0.05$).

CONCLUSION: No neurotransmitter changes are seen in PTSD caused by simple mental trauma, and the PCL-C scores do not associate with PTSD.

KEYWORDS: Post-traumatic stress disorders (PTSD), Neurotransmitter, Correlation

ÖZ

AMAÇ: Çalışmanın amacı, post travmatik stres bozukluğu (PTSB) hastalarında monoamin nörotransmitter seviyelerindeki değişiklikleri izlemek ve nörotransmitter seviyesinin PTSB şiddetiyle korelasyon gösterip göstermediğini saptamaktır.

YÖNTEM ve GEREÇLER: Herhangi bir fiziksel yaralanması olmayan toplam 15 PTSB hastası çalışmaya dahil edildi. Ayrıca 15 restorasyon öncesi hasta kontrol olarak kaydedildi; bu hastalar travmatik olaylar yaşamıştı ama PTSB durumu yoktu veya Post Travmatik Stres Bozukluğu Kontrol Listesi-Sivil versiyonu (PCL-C) puanı 12 puanın altındaydı. Norepinefrin, 5 hidroksitriptamin ve dopamin dahil plazma monoamin nörotransmitterlerinin seviyeleri enzim bağlantılı immünosorban testlerle test edildi.

BULGULAR: PTSB ve kontrol gruplarında monoamin nörotransmitter seviyeleri arasında önemli bir fark bulunmadı ($p > 0,05$). Korelasyon çalışması PTSB grubunda monoamin nörotransmitter seviyeleri ve PCL-C puanlarının istatistiksel olarak anlamlı korelasyon göstermediğini doğruladı ($p > 0,05$).

SONUÇ: PTSB durumunda basit mental travma ile bir nörotransmitter değişikliği görülmez ve PCL-C puanları PTSB ile ilişki göstermemektedir.

ANAHTAR SÖZCÜKLER: Post travmatik stres bozuklukları (PTSB), Nörotransmitter, Korelasyon

INTRODUCTION

Post-traumatic stress disorder (PTSD) is a stress-related disorder with serious clinical symptoms; PTSD is experienced by individuals facing unusually strong mental stress and could greatly damage mental health (14). This problem has become a prominent cause of concern; PTSD is aggravated by the occurrence of natural disasters and various accidents. After major natural disasters and other emergencies, the incidence of PTSD is relatively increased in a correlated population (21). The daily activities of affected patients usually change; these changes are accompanied by a significantly reduced quality

of life, excessive use of psychoactive substances, and other mental problems such as depression, anxiety, and phobia (17). The patients experience extreme pain and are vulnerable to concurrent co-morbidity, with significantly decreased social abilities. Breslau et al. (5) reported that 30% to 36%, 15%, and 10% to 13% of PTSD patients have major depression, obsessive-compulsive disorder, and panic disorder, respectively. The recovery rates of PTSD are significantly lower than those of other mental disorders. Furthermore, the pathogenesis of PTSD remains unclear. Imaging studies indicate that trauma or long-term stress may cause functional changes in neuroanatomic structures and neural networks

(18). The sympathetic nervous system (SNS), catecholamine (CA) system, the hypothalamic-pituitary-adrenal (HPA) axis, and the 5-hydroxytryptamine (5-HT) system are important for mood regulation, stress response, and awakening during PTSD occurrence and evolution (15, 25). This finding is related to the repeated manifestation of clinical PTSD, with high vigilance and fright vulnerability. However, the pathogenesis of these interactions remains unclear. To date, the neuroendocrine and immune changes during PTSD are considered the basis for changes in other organs. The current study was conducted to analyse the monoamine neurotransmitter levels of patients who experienced a traumatic event or their families with or without PTSD, as well as to explore their correlation with the disorder. This study aimed to provide a concrete basis for understanding the clinical pathogenesis of PTSD and its treatment. Previous studies investigated the relationship between neurotransmitters and PTSD and the results are controversial since the impact of injury in the patient's body was not completely excluded. The patients involved in this study had suffered purely mental trauma caused by PTSD without physical trauma to avoid interfering factors. Therefore, the aim of this study was to observe more accurately the relationship between neurotransmitter and PTSD and possibly provide a basis for exploring its pathogenesis.

MATERIAL and METHODS

Subjects

PTSD and control patients were all patients or their family members who experienced a traumatic event, and were treated in the Rehabilitation Psychology, Emergency Surgery, Neurosurgery, Burn, and Orthopaedics departments of the First Affiliated Hospital of the Shihezi University Medical College. Given that the determination of the neurotransmitter and immunological factors are easily affected by physical causes, patients with physical diseases or injuries were excluded in this study. Those who suffered from being beaten or raped, witnessing a traumatic event, or surviving a car accident, as well as the family members affected by a sudden accident, were included as the subjects. None of the patients were treated with any psychiatric medication, and had been affected by a traumatic event for more than one month. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Shihezi University Medical College. Written informed consent was obtained from all participants.

The following inclusion criteria were used: 1) Patients should meet the PTSD diagnostic criteria described in the fourth edition of the American Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (1). Response categories of "3" (Moderately) to "5" (Extremely) to the 17 items in the Civilian Version of the PTSD Checklist-(PCL-C) were determined as symptomatic. These values were summed in the total score. Patients with a total score ≥ 50 points, as determined by the clinical assessment two chief physicians, were included in the PTSD group. 2) Patients were over 18 years of age, regardless of gender. 3) Patients were of sound mind and willing to

answer the questionnaires and undergo scale investigations. 4) The patients had not used immunosuppressants or immunostimulants for at least six months. 5) The patients had not received any psychosedative medication in the past six months. 6) The patients had no history of alcohol or drug abuse. 7) The patients had no significant abnormal physical illness before the traumatic event. 8) The patients provided informed consent.

The exclusion criteria included: 1) Patients suffering from serious heart, brain, liver, or kidney disease as well as other serious physical illness, malnutrition, or obesity. 2) Patients with diseases of the endocrine, immune, or nervous systems, whether acute or chronic, as well as those with or mental retardation or other forms of mental illness. 3) Women who were pregnant, breastfeeding, or menstruating at the time. 4) Patients with a long-term history of alcoholism or drug dependence. 5) Patients with a history of PTSD or major depression.

Patients in the control group (pre-restoration patients without PTSD) had to meet all the above mentioned inclusion criteria, except for 1) Non-PTSD patients were clinically determined to belong to the pre-restoration group when they obtained less than 12 points in the PCL-C after they or their family members experienced traumatic events. These patients were then included in the control group.

Questionnaire

The severity of a patient's condition was assessed using the PCL-C questionnaire. This questionnaire is established according to the PTSD symptoms and standards, as described in the American Diagnostic and Statistical Manual of Mental Disorders (fourth edition, DSM-IV). The questions confirmed the corresponding key symptoms of PTSD.

The 17 items in the PCL-C were used to assess the three symptom clusters, namely, relief, numbness and avoidance, and excessive reaction. Respondents indicated how much they had been bothered by a symptom over the past month using a 5-point (1 to 5) scale. Responses ranged from 1 for "Not at All" to 5 for "Extremely" in this scale. A patient response from "3" to "5" (equivalent to "Moderately" or higher) can be regarded as symptomatic. The PCL-C score was included in the total score. The scale consistency coefficient ranged from 0.188 to 0.194; the retest reliability was 0.183 to 0.188. The correlation coefficients of anxiety, depression, and phobia for SCL-90 were all greater than 0.173, and the consistency of the diagnosis with DSM-IV was more than 90% (7).

A total of 15 PTSD patients (4 males and 11 females, aged 24 to 60 y) met the inclusion criteria and had a total PCL-C score of 52 to 79 points (62 ± 8 points). This included patients who had experienced the disorder for two months to three years. Another set of 15 non-PTSD patients (11 males and 4 females, aged 24 to 67 years) met the inclusion criteria and were included in the control group. Written informed content was obtained from each patient. No significant differences were found between the two groups based on the social

demographic characteristics of age, educational attainment and marriage ($p > 0.05$) except for gender (Table I).

Determination of Neurotransmitters

Briefly, 3 ml cubital venous blood was collected from each patient between 8:00 to 9:00 AM. The female patients were not experiencing their menstruation. Blood samples were placed at room temperature for 30 min and then centrifuged at 2500 rpm for 10 min. Plasma was transferred into EP tubes and stored at -70 °C. After sample collection, plasma was used in sandwich ELISA tests to detect the monoamine neurotransmitter levels. The ELISA detection kits of norepinephrine (NE), 5-HT, and dopamine (DA) were procured from China Shanghai Westang Bio-tech Co., Ltd. The tests for all samples were performed in duplicate.

Statistical Analysis

All results were analyzed using the SPSS (version 17.0) software (Chicago, IL, USA). Measurement data were presented as mean \pm standard deviation (SD). The parametric t-test and nonparametric rank-sum test were used for the populations with and without normal distribution, respectively. Numbered data were described in terms of their frequency (percentage) and compared using the χ^2 test (Fisher's exact test). The correlation between two groups was analysed using the

Pearson or Spearman correlation test for data with or without a normal distribution, respectively. Values with $p < 0.05$ were considered statistically significant.

RESULTS

Test of Normality

Given the small sample sizes of the two treatment groups, the test of normality was performed before the actual comparison. The measured values of NE and 5-HT were both found to follow a normal distribution in both groups ($p > 0.05$); by contrast, the values of dopamine in either group did not fit the normal distribution (Table II).

Monoamine Neurotransmitter Levels

The levels of monoamine neurotransmitters in the two groups were presented as the mean \pm SD and subsequently compared using t-tests. No significant differences were observed in the levels of plasma NE and 5-HT between the PTSD and the control groups ($t = 0.518, p = 0.608$ and $t = 0.199, p = 0.844$, respectively; Table III). Based on the non-normality of the plasma dopamine level, this characteristic was compared using the nonparametric rank-sum test. Our results showed that the detected values of dopamine in the two groups were not significantly different ($p > 0.05$; Table IV).

Table I: Demographic Data of the Two Groups

Items		PTSD group cases (%)	Control group cases (%)	t / χ^2	p value
Age (years)		43 \pm 10	39 \pm 12	1.046	0.305
Gender	M	4 (26.7)	11 (36.7)	6.533	0.027*
	F	11 (73.3)	4 (63.3)		
Education	Primary	6 (40.0)	3 (20.0)	1.577	0.455
	Middle	3 (20.0)	5 (33.3)		
	Secondary and higher	6 (40.0)	7 (46.7)		
Marriage	Single	1 (6.7)	3 (20.0)	5.045	0.080
	Married	8 (53.3)	11 (73.3)		
	Lost spouse	6 (40.0)	1 (6.7)		

Age was shown as mean \pm SD. * $p < 0.05$ indicates a significant difference.

Table II: Test of Normality of the Monoamine Neurotransmitters' Levels

Parameters	Groups	Kolmogorov-Smirnov ^a	
		Statistics	p values
NE	PTSD	0.214	0.063*
	Control	0.148	0.200*
5-HT	PTSD	0.122	0.200*
	Control	0.188	0.163*
Dopamine	PTSD	0.261	0.007
	Control	0.250	0.012
PCL-C	PTSD	0.170	0.200*

* $p > 0.05$ indicates that the data were consistent with a normal distribution.

Table III: Comparisons of Plasma NE and 5-HT Between the Two Groups

Parameters	PTSD group (n=15)	Control group (n=15)	t value	p value
NE (pg/ml)	1324.79±995.22	1171.88±560.79	0.518	0.608
5-HT (ng/ml)	825.61±154.11	813.87 ±168.61	0.199	0.844

Data were shown as mean±SD.

Table IV: Comparisons of Plasma Dopamine Between the Two Groups

Parameters	Groups	Sum Rank	Mann-Whitney U	Wilcoxon W	Z	p value
Dopamine	PTSD (n=15)	15.90	106.5	226.5	-0.249	0.803
	Control (n=15)	15.10				

Correlation Test

The correlation tests of plasma NE and 5-HT with the PCL-C scores were performed using the Pearson correlation test. We found that neither the plasma NE nor 5-HT were significantly correlated with the PCL-C scores in the PTSD group ($p > 0.05$). Correlation tests of the dopamine level with the PCL-C scores was conducted using the Spearman correlation test. The plasma dopamine level was not significantly correlated with the PCL-C scores in the PTSD group ($p > 0.05$).

DISCUSSION

Our study showed that no significant differences were present in the monoamine neurotransmitter levels (5-HT, NE, and dopamine) of the PTSD and control groups. This result was inconsistent with most of the previous studies. For example, Young and Breslau (24) reported that urine CA levels from PTSD in 69 communities were significantly higher than those of the traumatised non-PTSD group and a non-traumatised control group. In their study, the PTSD symptoms were positively correlated with the high CA levels. Hamner (10) found that plasma DA levels in some patients with PTSD may likewise increase under non-stimulated conditions. Stein (20) found that the brain 5-HT system is associated with the treatment of PTSD. Liu et al. (16) studied an animal model of PTSD. The over-expression of the 5-HT_{1A} receptor (5-HT_{1A}R) was found to induce PTSD and other related alertness behaviours, which is similar to the results of Zhang et al. (26). Both Brady et al. (4) and Krystal et al. (13) observed that NE is over-expressed in the hippocampus and amygdala of the brain during chronic PTSD.

In this study, the three biological indicators were not correlated with the PCL-C score. However, the NE levels in urine and cerebrospinal fluid were remarkably increased in the veterans suffering from PTSD, as compared with those without PTSD (23). Moreover, this elevated level was positively correlated with the clinician-administered PTSD scale (CAPS) (23). Young and Breslau (24) reported that the high level of CA has a positive relationship with the PTSD symptoms. The difference between our findings and the previous reports may result from the different scales, determination methods, sample sizes, and other factors.

Our results probably did not agree with most of the previous studies because of several factors. First, the sample size in this study was relatively small. The incidence of PTSD after a traumatic event is only 8%. However, a large sample size could reduce the sampling error as much as possible to obtain more accurate results. Second, the gender difference has a demographically significant effect on the comparison. For example, more females than males suffer from PTSD after traumatic event, but less of the population does not have PTSD after traumatic event. This finding is consistent with the demographic data of previous studies on traumatic PTSD. Third, the patient state situation after a traumatic event may be affected by factors such as their physical and mental condition and the high rate of comorbidity. In addition, the sample collection time, measuring method and materials, as well as the duration and type of a traumatic event will affect the measured values. This is probably the reason of inconsistency of the results of this study with the previous studies.

Severe stress reactions may produce a series of neurological changes and affect the structural and functional disorder of the HPA axis and other relevant brain areas at multiple levels. Consequently, such disorders lead to dysfunction of the autonomic nervous system. Certain individuals with this form of early damage are more likely to develop PTSD if they suffer from stress when they become adults (8, 19, 22). Among the clinical symptoms of PTSD, patients present characteristic symptoms of "recurring experience"; "flashbacks" are common manifestations (3). Patients feel persistent anxiety and fear during the unending "flashbacks" and are highly alert. All these characteristics may be explained by corresponding theories based on neuroendocrine research. Haqeman et al. (11) found that catecholamine can mediate mental and neurological abnormalities after stress. Relevant animal studies (6, 9, 12) showed that mouse 5-HT_{2C} knockouts and 5-HT_{2C} receptor antagonists have apparent anxiolytic effects. By contrast, the long-term treatment with paroxetine (SSRIs) can reduce anxiety-like symptoms in rats with the one-way long stress. Several lines of evidence indicate that the abnormal release of 5-HT induced constant fear, irritability, excitement, pugnacity, and so on. Another study showed that the DA level in the urine and plasma of patients with PTSD is positively associated with the severity of PTSD (2).

In conclusion, body homeostasis is disrupted after the input of strong mental stimulation, thereby causing obvious mood changes, uncontrolled impulses, and aggressive behaviour. As PTSD progresses, the steady-state system can continuously release neurotransmitters and undergo adaptive adjustments to minimize the self-induced damage and return the levels back to the range within neuroendocrine control. The levels of two neurotransmitters were higher in PTSD patients than in the controls, but this difference was not significant.

This study showed that there was no significant difference between PTSD and neurotransmitters after mental trauma, suggesting that new idea and basis for drug treatment of PTSD should be studied. Our study excluded the patients with physical injuries and focused on those with psychological trauma. However, the exclusion criteria were not sufficient to produce significant results. In the subsequent studies, we would refine the study by expanding the sample size, defining the traumatic stressor types (such as classifying acute and chronic PTSD with the same or similar nature) and expanding the horizontal controls (PTSD and traumatic non-PTSD and healthy controls). Likewise, we will determine the biochemical indicators in the cerebrospinal fluid of the relevant populations to exclude metabolism-related peripheral interference factors. In this manner, more effective results and values might be obtained.

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