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Correlation research of serum substance P, CCK-8, and 5-HT values with depression levels in stroke survivors

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Abstract. – OBJECTIVE: This study aimed to investigate the correlation of serum octapeptide cholecystokinin-8 (CCK-8), substance P (SP), and 5-hydroxytryptryptamine (5-HT) values with depression levels in patients with post-stroke depression (PSD). It also aimed to explore the potential approach for the early diagnosis of PSD.

PATIENTS AND METHODS: A correlation research between patients' biochemical indicators and depression levels was performed among 70 stroke patients during hospitalization from June 2021 to February 2022. The 70 stroke patients were selected and divided into post-stroke depression and non-depression groups according to the Hamilton Depression Scale (HAMD) score. The concentrations of CCK-8, SP, and 5-HT in both groups were measured, and the relationship between the values of CCK-8, SP, 5-HT and the depression levels was analyzed.

RESULTS: Among the 70 stroke survivors, 35 were in the depression group and 35 were in the non-depression group. Significant differences were observed in the concentration of CCK-8, SP, and 5-HT between the patients in the depression and non-depression group ($p < 0.05$). Accompanied by an increase in the depression level, the SP value gradually increased, but the CCK-8 and 5-HT values gradually decreased. Spearman correlation analysis indicated that the order of the correlation between CCK-8, 5-HT, SP, and the depression levels was CCK-8 > SP > 5-HT.

CONCLUSIONS: All the CCK-8, SP and 5-HT values were correlated with the depression levels in stroke survivors. Furthermore, the correlation between CCK-8, SP, and post-stroke depression levels was higher than that of 5-HT, suggesting that the early diagnosis of PSD may be reflected more precisely through the detection of CCK-8, and SP values, thus providing potential priority for biochemical detection in the diagnosis of PSD.

Key Words:
Post-stroke depression, Octapeptide cholecystokinin, Serum substance P, 5-HT.

Introduction

Post-stroke depression (PSD) refers to a syndrome in which stroke survivors present a series of depressive and corresponding somatic symptoms, especially an affective disorder syndrome characterized by increased functional, cognitive and communicative disability, reduced quality of life, and increased mortality[1-3]. Therefore, depression after stroke negatively affects patient participation in rehabilitative practice and associated patient outcomes[4]. Patients with major depression have reported[5] higher levels of disability than those without depression, and a different study[6] carried out in Europe found that the degree of disability was related to the severity of depression in patients with major depressive disorder. PSD disease not only increases the economic burden of stroke patient's families, but also prolongs the hospital stay because of its insidious onset.

The early detection of depression after stroke is important to recommend an appropriate therapy for depressed patient. Scales such as the Hamilton Depression Rating Scale (HDRS)[7], Beck Depression Inventory[8], Montgomery Asberg Depression Rating Scale[9], Zung Depression Scale[10], and Geriatric Depression Scale[11] are used to evaluate depression in patients with stroke, and these tools may produce large deviation because the assessments depend on the doctor's subjective judgments[12]. The detection

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of biochemical indicators can be used for the objective evaluation of the depression level of the patient. Treatment with antidepressants and suitable anticoagulant may decrease the severity of the depression and improve functional status[13,14]. The levels of serum lipid were measured, and the result illustrated that the serum cholesterol levels were higher in patients with acute post-stroke depression (PSD) than in acute stroke patients without depression[15]. Serum lipid profiles, including high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, total cholesterol, Apo lipoprotein A1, and Apo lipoprotein B were measured in a group of acute ischemic stroke patients, and significant differences were observed[16] in HDL-C, and LDL-C levels between the PSD and non-PSD groups. 5-Hydroxytryptamine (5-HT) affected the depressive symptoms in patients[17]. Clinical studies[18] have shown bidirectional interactions within the brain-gut-microbiome axis. Therefore, the biochemical indicators of human circulatory system may have a potential correlation with the depression levels of stroke survivors. This study measured the serum substance P (SP), cholecystokinin-8 (CCK-8), and 5-HT values between depressed and non-depressed stroke survivors to investigate the correlation of these parameters with depression levels in patients with PSD.

Patients and Methods

Recruitment of Patients

Diagnosis of PSD is still straightforward in many cases, because several non-depressive neurologic stroke sequelae may resemble a symptom of depression. In the present study, 70 stroke survivors who were confined in our hospital from June 2021 to February 2022 were selected according to the diagnostic criteria of expert consensus on the clinical practice of post-stroke depression in China. Among the patients, 35 patients who were satisfied of the diagnostic criteria of depression were classified into the depression group, and the other 35 patients without depressive symptoms were classified into the non-depression group. This study was approved by the Ethics Committee of Shenzhen Dapeng New District Nan'ao People's Hospital, and written informed consents were obtained from all participants before the investigation.

Study Protocol

The values of the biochemical indicators of SP, CCK-8, and 5-HT were measured. Each patient in both groups was asked to draw 5 mL of venous blood on an empty stomach at 7-9 am on the day of the examination. The supernatant of the venous blood was collected after centrifugation at 3,000 rpm and stored in a refrigerator at -80°C for testing. The SP, CCK-8, and 5-HT values were measured using a microplate reader (model: MR-96A, Shenzhen Mindray Biomedical Electronics Co., Ltd, Shenzhen, China) *via* enzyme-linked immunosorbent assay.

All patients were evaluated through the Hamilton Depression Rating Scale (HAMD, 17 items)[19] for depression symptom assessment. The assessment was performed by an expert owning more than 10 years experiences with the use of the HAMD. The patient manifestation is divided into 4 levels according to the HAMD total scores of 8, 17 and 24 points, respectively. The patient was considered in a normal state at HAMD total scores of less than 8 points, mild depression with the HAMD total scores ranging between 8-17 points, moderate depression with the HAMD total scores ranging between 17-24 points, and severe depression with the HAMD total scores more than 24 points.

Statistical Analysis

All statistical analyses were performed using Statistical Package for Social Science (SPSS) 24.0 software (IBM Corp., Armonk, NY, USA). The mean-standard deviation ($x \pm s$) was used to represent the measurement data. Independent-samples *t*-test was used for the intergroup comparison, and the LSD-*t* test was used for multiple comparisons between the means of multiple samples. Paired *t*-test was used for intra-group comparison, Spearman correlation test was used for the correlation analysis between groups, and enumeration data were analyzed using χ^2 test. Statistical significance was considered at $p<0.05$.

Results

General Information of the Included Patients

Table I shows the general information of the depression group and non-depression group patients. A total of 20 males and 15 females with average age of (61.06 ± 13.75) years old were included in the depression group, while 23 males and 12

Table I. General information about patients in both groups.

	Gender		Age	Stroke type		Brain injury site	
	Male	Female		Ischemic	Hemorrhagic	Left	Right
Depression group	20 (57.2%)	15 (42.8%)	61.06 ± 13.75	23 (65.7%)	12 (34.3%)	15 (42.8%)	20 (57.2%)
Non depression group	23 (65.7%)	12 (34.3%)	63.06 ± 15.64	27 (77.1%)	8 (22.9%)	11 (31.5%)	24 (68.5%)
χ^2/t	0.245		0.568[a]		0.110	0.117	
p	0.469		0.578		0.297	0.330	

Parameter a means t-value, other means χ^2.

females with average age of (63.06 ± 15.64) years old were included in the non-depression group. The patients' stroke type and brain injury site were recorded. The *t*-test results demonstrated no significant difference in patients' gender, age, stroke type, and brain injury site.

Measurement of SP, CCK-8 and 5-HT Values

The patients were divided into non-depression, mild depression, moderate depression, and severe depression groups according to the HAMD scores. The results demonstrated significant differences in the SP, CCK-8, and 5-HT values between the patients in depression and non-depression group ($p<0.05$). Accompanied by a progressively higher levels of depression, the SP value gradually increased, whereas the CCK-8 and 5-HT values gradually decreased ($p<0.05$). The measure of SP, CCK-8, and 5-HT corresponding to depression levels are presented in Table II.

Correlation Between Serum CCK-8, SP, 5-HT, and PSD

Spearman correlation test was used to analyze the correlation between serum CCK-8, SP, 5-HT and PSD. The results indicated that CCK-8 is highly correlated with PSD, while 5-HT and SP are moderately correlated with PSD, as presented in Table III.

Discussion

Depressive symptoms were present in approximately 30% of all stroke survivors[20]. Early detection is an important prerequisite for the treatment of PSD. However, PSD is mostly insidious and is not easily noticed by clinicians, accompanying staff, and even patients themselves. HAMD is the most commonly used depressive symptom assessment scale in clinical practice. Its validity and evaluation time point still cannot meet the clinical needs. Literature has focused on the influencing factors of inflammatory mediator release and neurotransmitter changes, but less on the correlation between the serum SP, CCK-8, 5-HT values, and the PSD levels. This study aimed to explore the pathogenesis of PSD and explore the correlation of the biochemical indices in combination with the assessment scale score to improve the accuracy of the early diagnosis of PSD.

PSD is an organic affective disorder, which is caused by the imbalance of 5-HT, norepinephrine (NE), and dopamine (DA) system[21,22]. In the early 1990s, the brain-gut axis (central nerve - enteric nerve - brain-gut peptide) theory was gradually matured accompanying the development of neurogastroenterology. The brain-gut axis is a bidirectional channel between the central nervous system, neuroendocrine, autonomic, enteric, and immune systems[23]. The human body makes irritable responses caused by external stimuli and emotional changes. The signal generated from the central nervous system of the brain regulates gastrointestinal secretions, blood supply, and gastrointestinal secretions through the brain-gut peptides released by the enteric nervous system. The central nervous system makes adaptive adjustment after receiving the stimuli from the receptors in the gastrointestinal tract. Both SP and CCK are brain-gut peptides, which are widely distributed in the central and gastrointestinal systems. Neurokinin 1 (NK1), which is a receptor for SP, has antidepressant activity and is involved in the pathological and physiological processes of affective disorders[24]. Sulfated CCK-8 is the main form of biologically active CCK in the brain. Mice can produce a depressive behavior after an injection with CCK-8 in the medial prefrontal lobe. This finding[25] suggests that reduced neuronal activity may be involved in the pathogenesis of depression. Therefore, changes in SP and CCK-8 values may be the important pathological and physiological reasons for the onset of PSD.

Table II. Comparison of CCK-8, 5-HT and SP values among the stroke patients with different levels of depression.

		Number	CCK-8 (pg/mL)	5-HT (ng/mL)	SP (pg/mL)
Non-depression		35	64.80 ± 39.19	105.82 ± 71.98	34.60 ± 20.47
Depression	Mild	20	34.13 ± 10.12	66.51 ± 25.18	45.62 ± 8.96
	Moderate	5	19.18 ± 8.33	54.45 ± 12.38	79.34 ± 46.02
	Severe	10	12.88 ± 8.58	46.44 ± 7.90	118.15 ± 73.43
	F		11.873	4.772	17.795
	p		0.000	0.005	0.000

Table III. The Ct value of YAP1 was detected by qRT-PCR after transfection with si-YAP1.

	CCK-8	5-HT	SP
R	-0.820	-0.620	0.682
p	0.000	0.000	0.000

R represents the correlation ratio. Range (0.8-1.0) means highly correlation; range (0.6-0.8) means moderate correlation.

The trend of the SP, CCK-8, and 5-HT values accompanied by the depression levels in the stroke survivors is shown in Figure 1. The experimental results indicate that the SP value is positively correlated with depression levels, while CCK-8 and 5-HT are negatively correlated with depression levels. The probable mechanism is that the neurobiological disorder caused by cerebral hypoperfusion leads to less secretion of NE and 5-HT, and decreased concentration of 5-HT and CCK-8. However, both ischemic and hemorrhagic strokes alter the internal structure of the brain and damage the aggregation region of SP and its receptor NK1. It therefore disrupts the synthesis, storage, transport, and release of SP, leading to an abnormal increase of the SP value.

This study analyzed the correlation between SP, CCK-8, and 5-HT values and depression levels in stroke survivors. Results show that the correlation coefficients between CCK-8 and SP and the depression levels are higher than that of 5-HT, suggesting a higher sensitivity to diagnose early PSD occurrence by detecting CCK-8 and SP values. The potential explanation is that PSD patients commonly suffers from loss of appetite, and its gastrointestinal function is affected, resulting in an emergency response of brain-gut peptide. Therefore, the value of SP and CCK-8 can be much more sensitive to PSD occurrence.

The results demonstrate that the values of SP, CCK-8, and 5-HT are closely related to the severity of depression in patients with PSD. The potential benefit of early diagnosis of PSD can be expected by measuring the values of SP, CCK-8, and 5-HT. However, the sample capacity in the current study is limited, and many underlying diseases in patients may generate a certain deviation. An expanded sample size research will be carried out in further studies.

Conclusions

PSD is easily neglected because of its hidden symptoms, and it leads to potential high-risk of emergency occurrence for stroke survivors. The accurate assessment of PSD is essential to rehabilitative intervention, but traditional scales are trapped in user subjectivity. In the present study, the experimental results demonstrated that severe PSD is positively correlated with the SP value but negatively correlated with the CCK-8 and 5-HT values. Meanwhile, compared with 5-HT, the value of SP and CCK-8 can be much more sensitive to PSD occurrence.

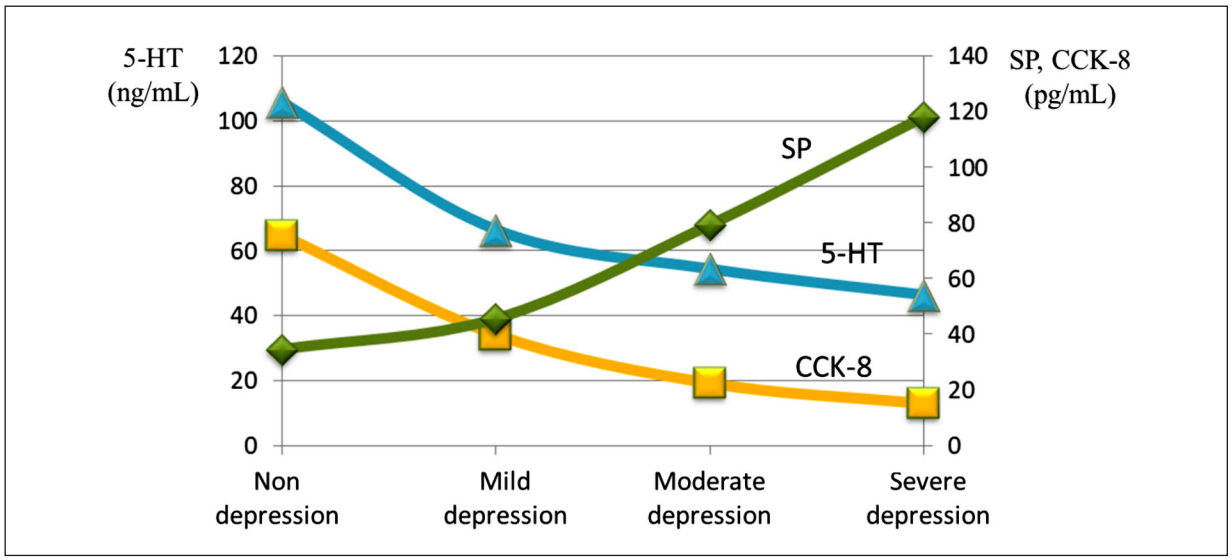

Figure 1. The correlation between SP, 5-HT, CCK-8 average values and depression levels in stroke survivors.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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Ethics Approval
This study was approved by the Ethics Committee of Shenzhen Dapeng New District Nan'ao People's Hospital.

Informed Consent
Written informed consents were obtained from all participants before the investigation.

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Availability of Data and Materials
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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