

Original Article

Correlation between serum neuropeptide Y, substance P, and 5-HT expression and anxiety and depression in constipation-predominant irritable bowel syndrome patients

Lianrong Guo¹, Yanying Zhao², Bin Liu¹

¹Department of Gastroenterology, ²Physical Examination Center, The Second Hospital of Shandong University, Jinan City 250033, Shandong Province, P. R. China

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Abstract: Objective: The aim of this study was to investigate the correlation of serum neuropeptide Y, substance P, and 5-hydroxytryptamine (5-HT) expression with anxiety and depression in patients with constipation-predominant irritable bowel syndrome (C-P IBS). Methods: Between January 2016 and May 2017, 68 patients with C-P IBS, diagnosed and treated in The Second Hospital of Shandong University, were enrolled in this study (observation group). Additionally, 68 concomitant healthy persons receiving physical examinations were enrolled as controls (control group). Serum neuropeptide Y, substance P, and 5-HT expression of all eligible participants were measured with application of Enzyme-linked immunosorbent assay (ELISA). Anxiety and depression of the two cohorts were evaluated using the Self-Rating Anxiety Scale (SAS) and Self-Rating Depression Scale (SDS). Any correlation of serum neuropeptide Y, substance P, and 5-HT expression with anxiety and depression were elucidated by Pearson's correlation analysis. Results: Compared to the control group, serum substance P expression of the observation group was significantly increased but serum neuropeptide Y and 5-HT expression were remarkably reduced in the observation group (all $P < 0.05$). SAS and SDS scores in the observation group were also considerably higher (both $P < 0.05$). Serum substance P levels in C-P IBS patients with anxiety and depression were strikingly higher, but serum neuropeptide Y and 5-HT levels were remarkably lower than those without anxiety and depression (all $P < 0.05$). Among C-P IBS patients, there was a negative correlation of serum neuropeptide Y levels to SAS and SDS scores ($r = -0.624$, $P = 0.012$; $r = -0.714$, $P = 0.005$), positive correlation of serum substance P levels to SAS and SDS scores ($r = 0.715$, $P = 0.002$; $r = 0.675$, $P = 0.007$), and negative correlation of serum 5-HT levels to SAS and SDS scores ($r = -0.784$, $P = 0.001$; $r = -0.762$; $P = 0.003$). Conclusion: Neuroendocrine changes occurred in most C-P IBS patients. Abnormal levels of serum neuropeptide Y, substance P, and 5-HT might correlate with anxiety and depression in C-P IBS patients.

Keywords: Constipation-predominant irritable bowel syndrome, neuropeptide Y, substance P, 5-hydroxytryptamine, anxiety and depression, correlation

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain or discomfort and constipation, without overt and specific morphological changes and biochemical abnormalities [1, 2]. Most IBS patients present with constipation, hence, it is known as constipation-predominant (C-P) IBS. C-P IBS may contribute to anxiety and depression and affect patient quality of life. However, the pathogenesis of the condition

remains unclear [3, 4]. Current research indicates that onset and development of C-P IBS are closely related to abnormal mental and psychological factors. C-P IBS patients frequently have anxiety and depression, suggesting that there may be common underlying pathological mechanisms between them [5, 6]. Interactions between the central nervous system and enteric nerves are commonly achieved through secreting neurotransmitters or gastrointestinal hormones (including neuropeptide Y, substance P, and 5-hydroxytryptamine (5-HT)), transmit-

ting them among the enteric nervous system, gastrointestinal effector cells, and central nervous system [7]. Neuropeptide Y, substance P, and 5-HT have been found to be implicated in the regulation of gastrointestinal functions. Disorders of their secretion are one of the crucial mechanisms leading to anxiety and depression [8, 9]. Nevertheless, few studies have investigated the correlation of serum neuropeptide Y, substance P, and 5-HT levels with anxiety and depression in patients with C-P IBS. Therefore, the purpose of this present study was to investigate the association of serum neuropeptide Y, substance P, and 5-HT levels with anxiety and depression in patients with C-P IBS.

Materials and methods

Data collection

Patients: From January 2016 to May 2017, 68 patients with C-P IBS, diagnosed and treated in The Second Hospital of Shandong University, were recruited into this study and assigned to the observation group. Males made up 70.59% (48/68) of patients and 29.41% (20/68) were female, with a mean age of (45.40±4.80) years. Additionally, 68 healthy volunteers, after physical examination, were also recruited and assigned to the control group. Males made up 52.94% (36/68) of these patients and 47.06% (32/68) were female, with a mean age of (41.24±2.81) years. Mild differences were seen in age and gender between patients in the observation group and healthy subjects in the control group (all $P>0.05$).

Inclusion criteria and exclusion criteria: Patients older than 18 years were enrolled in this study if they met clinical diagnostic criteria for C-P IBS (a subtype of functional gastrointestinal disorders); if they had recurrent abdominal pain or discomfort persistent for more than 6 months, monthly constipation and altered stool traits, and if abdominal pain or discomfort improved after defecation in the previous 3 months; if no significant or specific morphological changes and biochemical abnormalities were detected by gastroscopy, colonoscopy, and barium meal examinations of the gastrointestinal system in the previous 6 months [10].

Patients were excluded if they had constipation, abdominal pain, or discomfort due to

organic disease; if they were women in pregnancy or lactation; if they had a history of abdominal surgery; if they had severe major organ dysfunction syndrome involving the heart, liver, and kidneys; if they had mental or cognitive dysfunction, neurological disorder, autoimmune disease, or thyroid dysfunction.

The Hospital Ethics Committee approved this study and all patients submitted written informed consent.

Study methods

Symptom scoring criteria: Criteria for rating and scoring clinical symptoms of IBS, specified in the Rome III classification system, were utilized to assess symptoms of abdominal pain, abdominal distension, abnormal defecation, and character of stool in patients with C-P IBS. Each symptom was scored in terms of severity and attack frequency. Severity of the condition ranged from no symptom, mild, overt, to severe symptom, scoring 0, 1, 2 and 3 points, respectively. Frequency of IBS attacks varied from less than twice a week, 2-5 times a week, to more than 5 times a week, scoring 0, 1 and 2 points, respectively. Higher scores indicated more severe symptoms [11].

Determination of serum neuropeptide Y, substance P, and 5-HT levels: Enzyme-linked immunosorbent assay (ELISA) was utilized to detect levels of neuropeptide Y, substance P, and 5-HT in the serum of all participants. Details are as follows: 5 mL of venous blood was drawn from each participant in a fasting state, placed in an anticoagulation tube, and centrifuged at 3000 r/min for 10 minutes. Subsequently, supernatant was transferred into a test tube and stored at -20°C. ELISA kits for detection of neuropeptides Y, substance P, and 5-HT were purchased from R&D science, USA, and procedures were conducted in accordance with manufacturer instructions.

Rating scales: Symptoms of anxiety and depression of patients were rated by two senior physicians using the Self-Rating Anxiety Scale (SAS) and Self-Rating Depression Scale (SDS).

SAS consisted of 20 items, with each item scoring on a subscale of 1-4 (1 = a little of the time, 2 = some of the time, 3 = good part of the time, and 4 = most of the time). Scores of the 20

Table 1. Serum neuropeptide Y, substance P, and 5-HT expression

Variables	Case	Neuropeptide Y (µg/mL)	Substance P (µg/mL)	5-HT (ng/mL)
Control group	68	8.68±3.00	3.56±1.03	177.15±28.63
Observation group	68	5.72±2.06	5.72±1.28	123.26±25.37
t value		2.815	3.940	2.748
P value		0.010	0.001	0.029

Note: 5-HT denotes 5-hydroxytryptamine.

Table 2. SAS and SDS scores of the two groups (score)

Variables	Case	SAS score	SDS score
Control group	68	24.35±2.52	25.17±2.47
Observation group	68	52.15±4.43	50.64±6.48
t value		15.415	14.825
P value		<0.001	<0.001

Note: SAS denotes the Self-Rating Anxiety Scale; SDS, the Self-Rating Depression Scale.

items were summed up, calculated, and converted into standard scores. According to the Chinese normative standard, 50 points was considered as the cutoff value. Less than 50 points expressed no anxiety and equal to or greater than 50 points expressed anxiety, with higher scores indicating more severe anxiety. Scores for mild, moderate, and severe anxiety fell into three respective ranges of 50-59, 60-69, and 70 or above [12].

The SDS is a scale of 20 items, with each item scoring on a 4-point subscale (1 = seldom, 2 = sometimes, 3 = often, and 4 = persistent, respectively). Scores of the 20 items were summed and converted into standard scores. Based on the Chinese normative standard, 53 points was defined as the cutoff value, with higher scores indicating more severe depression. Scores for mild, moderate, and severe depression fell into three respective ranges of 53-62, 63 to 72, 72 or above [13].

Outcome measures

Primary outcomes included serum neuropeptide Y, substance P, and 5-HT levels of patients with C-P IBS and healthy controls, as well as differences in SAS and SDS scores between the two cohorts. Secondary outcome was the correlation of serum neuropeptide Y, substance P, and 5-HT levels with SAS and SDS scores in patients with C-P IBS.

Statistical analysis

All statistical data were processed using SPSS software, version 18.0. Quantitative data are described as mean ± sd and independent samples t-tests were used for comparison between groups. Count data are presented as percentages and Chi-square tests were employed for comparison

between groups. Correlation between serum neuropeptide Y, substance P, and 5-HT levels with anxiety and depression in patients with C-P IBS were clarified using Pearson's correlation analysis. P<0.05 was deemed as statistically significant.

Results

Serum neuropeptide Y, substance P, and 5-HT levels in the observation group and the control group

The observation group had substantially higher levels of substance P, but considerably lower levels of serum neuropeptide Y and 5-HT than the control group (all P<0.05, **Table 1**).

SAS and SDS scores of the two groups

Remarkably higher SAS and SDS scores were noted in patients in the observation group than in healthy subjects of the control group (P<0.05, **Table 2**).

Serum neuropeptide Y, substance P, and 5-HT levels in C-P IBS patients associated with/without anxiety and depression

The basic data differed, insignificantly, between C-P IBS patients with anxiety and depression and those without anxiety and depression (P>0.05, **Table 3**). C-P IBS patients with anxiety and depression had considerably lower serum neuropeptide Y and 5-HT levels, but significantly higher substance P than those without anxiety and depression (all P<0.05, **Table 4**).

Correlation of serum neuropeptide Y, substance P, and 5-HT levels with SAS and SDS scores

Pearson's correlation analysis indicated, among patients with C-P IBS, there was a negative correlation of levels of neuropeptide Y (r =

Table 3. Basic data of C-P IBS with/without anxiety and depression

Variable	Case	Male/ Female	Age (year)	IBS score	COD (year)	SAS score	SDS score
C-P IBS patients without anxiety and depression	26	17/9	42.50±4.2	19.62±2.45	6.54±3.82	32.45±2.61	33.18±2.58
C-P IBS with anxiety and depression	42	31/11	47.20±5.1	19.83±2.79	7.28±4.63	64.35±4.73	61.45±5.93
t/ χ^2 value		0.549	1.232	0.083	0.214	13.726	16.105
P value		0.459	0.285	0.938	0.841	<0.001	<0.001

Note: C-P IBS denotes constipation-predominant irritable bowel syndrome; IBS, irritable bowel syndrome; COD, course of disease; SAS, the Self-Rating Anxiety Scale; SDS, the Self-Rating Depression Scale.

Table 4. Serum neuropeptide Y, substance P, and 5-HT expression in C-P IBS patients associated with/without anxiety and depression

Variable	Case	Neuropeptide Y ($\mu\text{g/mL}$)	Substance P ($\mu\text{g/mL}$)	5-HT (ng/mL)
C-P IBS patients without anxiety and depression	26	7.65±1.64	4.39±1.11	149.77±24.36
C-P IBS with anxiety and depression	42	4.52±1.23	6.54±1.42	106.85±13.77
t value		3.755	3.580	3.757
P value		0.004	0.003	0.004

Note: 5-HT denotes 5-hydroxytryptamine; C-P IBS, constipation-predominant irritable bowel syndrome.

Table 5. Correlation of serum neuropeptide Y, substance P, and 5-HT expression with SAS and SDS scores

Marker	SAS		SDS	
	r value	P value	r value	P value
Neuropeptide Y	-0.624	0.012	-0.714	0.005
Substance P	0.715	0.002	0.675	0.007
5-HT	-0.784	0.001	-0.762	0.003

Note: 5-HT denotes 5-hydroxytryptamine; SAS, the Self-Rating Anxiety Scale; SDS, the Self-Rating Depression Scale.

-0.624, P = 0.012; r = -0.714; P = 0.005) and 5-HT (r = -0.784, P = 0.001; r = -0.762; P = 0.003) in serum with SAS and SDS scores, but a positive correlation of substance P levels with SAS and SDS scores (r = 0.715, P = 0.002; r = 0.675, P = 0.007, **Table 5, Figures 1-3**).

Discussion

The etiology and pathogenesis of C-P IBS is complicated and may be implicated in brain-intestinal interactions and neuroimmunological mechanisms [14]. Increasing evidence has demonstrated that anxiety and depression may lead to abnormal gastrointestinal neuromodulation in patients with C-P IBS and further exacerbate anxiety and depression [15]. A study on the pathogenesis of C-P IBS has shown that anxiety and depression result in access to vari-

ous bioactive media pathways, which increase sensitivity of the gastrointestinal tract, change the smooth muscle contraction and dilation, and contribute to abnormal smooth muscle motility in the intestinal tract [16]. SAS and SDS are currently widely-recognized and authoritatively objective and quantitative tools for assessing the psychological status of patients. In the current study, SAS and SDS scales were utilized to quantify the severity of anxiety and depression in patients with C-P IBS. Results showed that SAS and SDS scores of patients in the observation group were remarkably higher than those in the control group (P<0.05), implying that anxiety and depression were present in patients with C-P IBS. These results align well with results reported by Liu et al. [17]. In the current study, gastrointestinal symptoms were closely associated with anxiety and depression in patients with C-P IBS. This might be attributed to the fact that there are common pathological mechanisms between C-P IBS and anxiety and depression, such as abnormal neurotransmitter function.

Neuropeptide Y is a polypeptide primarily expressed in the brain and gastrointestinal tissues. It is recognized as a gut hormone that plays a crucial role in regulating gastric acid secretion and acts to stimulate or inhibit the central nervous system [18]. Westrin et al. reported that substantially lower serum neuro-

Neuropeptide Y, substance P and 5-HT expression in serum, anxiety and depression

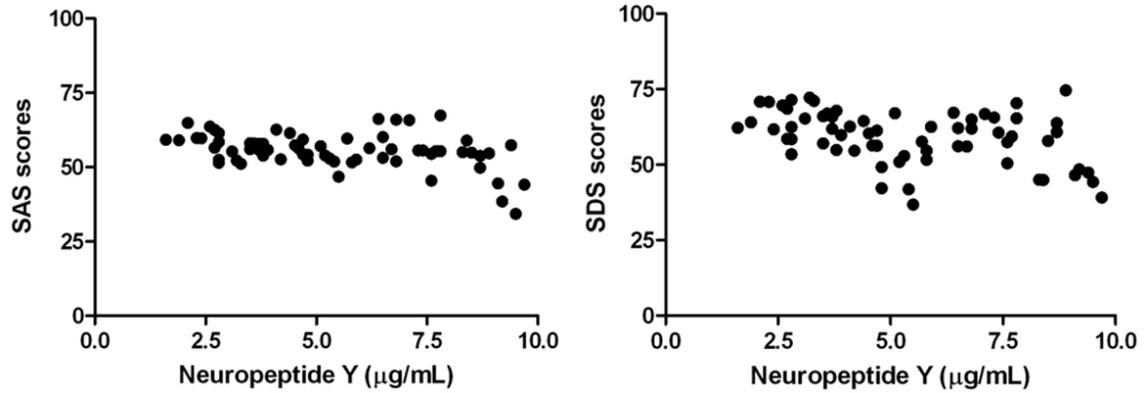


Figure 1. Scatterplot analysis on correlation of serum neuropeptide Y levels with SAS and SDS scores. Note: SAS denotes the Self-Rating Anxiety Scale; SDS, the Self-Rating Depression Scale.

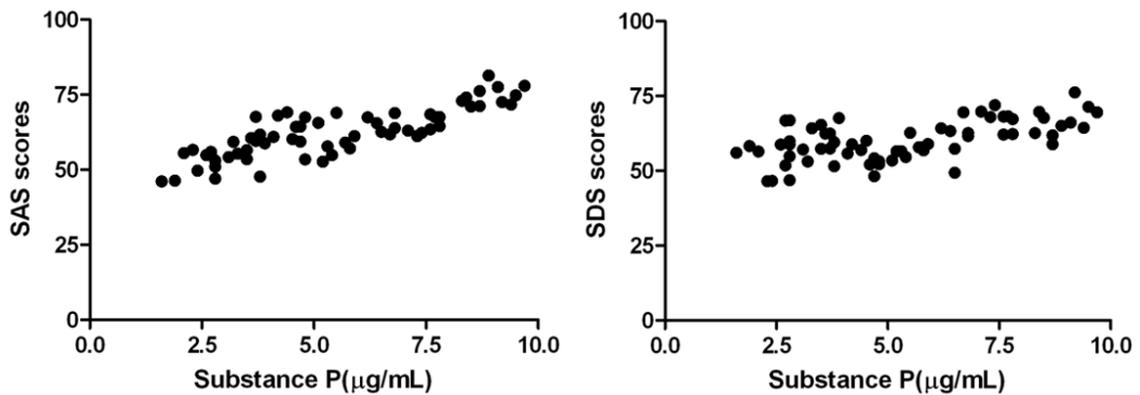


Figure 2. Scatterplot analysis on correlation of substance P levels with SAS and SDS scores. Note: SAS denotes the Self-Rating Anxiety Scale; SDS, the Self-Rating Depression Scale.

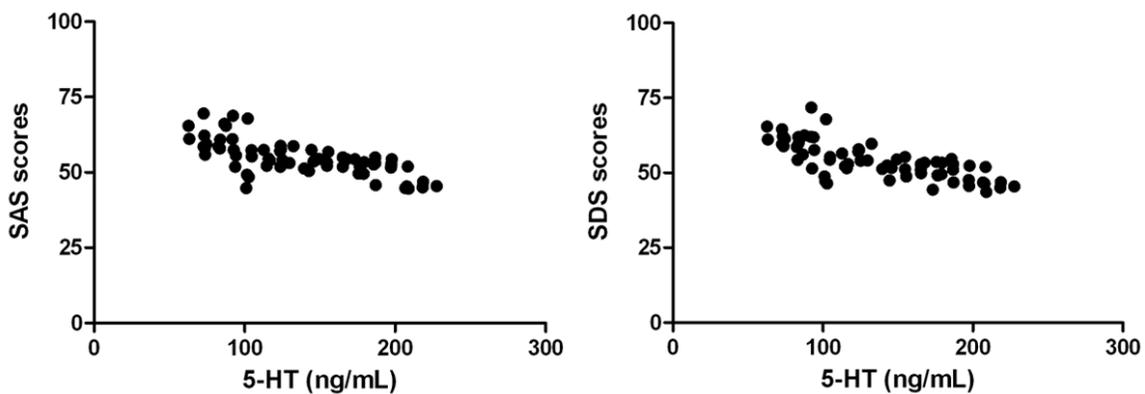


Figure 3. Scatterplot analysis on correlation of 5-HT levels with SAS and SDS scores. Note: 5-HT denotes 5-hydroxytryptamine; SAS, the Self-Rating Anxiety Scale; SDS, the Self-Rating Depression Scale.

peptide Y levels in depressive patients predisposed them to suicide more than healthy controls [19]. Neuropeptide Y is a neuropeptide

rich in humans, exerting endogenous sedative, hypnotic, anxiolytic, and antidepressant effects [20]. In the present study, it was found that

strikingly lower serum neuropeptide Y levels were observed in C-P IBS patients than in controls and C-P IBS patients with anxiety and depression had remarkably lower serum neuropeptide Y levels than those without anxiety and depression. Pearson's correlation analysis revealed that serum neuropeptide Y levels were negatively correlated with patient anxiety and depression. This suggests that abnormal levels of serum neuropeptide Y may be implicated in the presence of anxiety and depression in patients with C-P IBS.

Substance P is also a neuropeptide substance, mostly distributed in regions associated with mood regulation in the central nervous system. Substance P is an excitatory neurotransmitter acting as a trigger of anxiety, depression, and arousal [19]. Previous research has indicated that serum substance P levels are higher in patients with depression than those of controls [21]. Substance P is distributed in the gastrointestinal tract and implicated in regulation of intestinal peristalsis and gastric contraction by stimulating smooth muscle contraction and dilation in the gastrointestinal tract [22]. The results of the current study revealed that patients with C-P IBS had substantively higher serum substance P levels than healthy controls, and C-P IBS patients with anxiety and depression had remarkably higher serum substance P levels than those without anxiety and depression. Pearson's correlation analysis identified a positive correlation between serum substance P levels and anxiety and depression in patients. This indicates that abnormal levels of serum substance P partly contribute to the development and progression of anxiety and depression in patients with C-P IBS.

5-HT is extensively distributed in the gastrointestinal tract and central nervous system. A decisive neurotransmitter of the brain-intestine axis, 5-HT exerts multiple physiological functions including involvement in gastrointestinal motility and regulation of cognition, emotion, and memory. In recent years, increasing attention has been paid to exploring the actions of 5-HT in the pathogenesis of IBS and anxiety and depression. Elevated 5-HT levels act on receptors, contributing to changes in intestinal perception, secretion, and movements as well as high sensitivity in visceral afferents and the enteric nervous system, activating multiple

neuroactive substances and leading to chemical signal disorders of the brain-gut axis [23]. In the present study, remarkably lower serum 5-HT levels were found in C-P IBS patients than in healthy controls. Negative correlation was found between serum 5-HT levels and SAS and SDS scores in patients ($P < 0.05$), suggesting that 5-HT dysfunction may be implicated in the pathogenesis of constipation of IBS patients with anxiety and depression.

In conclusion, this study showed abnormal levels of neuropeptide Y, substance P, and 5-HT in serum present in patients with C-P IBS. They were significantly associated with anxiety and depression in patients. Monitoring serum neuropeptide Y, substance P, and 5-HT levels is beneficial to the evaluation and management of C-P IBS. However, there were some limitations to this study, including a small sample size and the fact that it was a single-center study. Additional multi-center studies with larger sample sizes are necessary to further confirm the results, clarifying the pathogenesis of anxiety and depression in patients with C-P IBS from the perspective of molecular mechanisms.

Disclosure of conflict of interest

None.

Address correspondence to: Bin Liu, Department of Gastroenterology, The Second Hospital of Shandong University, No. 247, Beiyuan Street, Jinan City 250033, Shandong Province, P. R. China. Tel: +86-0531-88197777; Fax: +86-0531-88197777; E-mail: BinLiu78@163.com

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