Original Article

Mucosal Serotonin Reuptake Transporter Gene Expression and Serum Level among Iraqi Patients with Irritable Bowel Syndrome

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Abstract

Background: A serotonin reuptake transporter is a protein that regulates serotonin levels in the body. Altered expression of the serotonin transporter gene and changes in serum levels of serotonin have been linked to irritable bowel syndrome (IBS). Research suggests that differences in serotonin transporter expression and serum levels may be associated with the development of IBS and its symptoms. **Objective:** This study aimed to measure selective serotonin reuptake transporter (SERT) expression level and SERT serum concentration among patients and control and find out the relationship between the severity of IBS syndrome and those levels. **Materials and Methods:** The present study included 100 specimens divided into two groups: 50 healthy control subjects and 50 IBS patients. Each patient and control had provided 2 mL of venous blood for the measurements of SERT level with serum, while 27 biopsy samples of patients and 16 mucosal samples of control were collected from patients with IBS. In this study, we used real-time polymerase chain reaction to quantify gene transcripts of SERT. Additionally, the SERT serum level was measured using enzyme-linked immunosorbent assay. These molecular analyses provide insights into the roles and interactions of these molecules, offering a better understanding of their functions in the biological system under investigation. **Results:** Data regarding serotonin expression was found to be nonnormally distributed; accordingly, they were expressed as median and rand (beside mean ± SD) and analyzed with a nonparametric Mann–Whitney *U* test. The relative median expression of the serotonin transporter gene was 1.05 folds (range 0.12–200 folds) in patients compared to 0.5 folds (range 0.07–12.43 folds) in control with no significant difference. The median serum level of the serotonin transporter in IBS patients was 12.8 pg/mL (range 0.6–36.13 pg/mL) with a mean lower than that of controls (median = 11.89 pg/mL, range = 9.44–33.5 pg/mL) with no significant difference. **Conclusion:** According to the findings of the current study, high SERT gene expression, as well as its serum level, is related to visceral hypersensitivity that leads to worsened development of IBS symptoms.

Keywords: Enzyme-linked immunosorbent assay (ELSA) test, gastrointestinal tract, visceral hypersensitivity

Introduction

Irritable bowel syndrome (IBS) is a disorder of gut–brain interaction that affects approximately 4% of the global population. However, estimates vary based on study methodology.[1] IBS patients often differ from IBD patients, who experience symptoms such as altered stool forms, constipation, bloating, and abdominal pain. They may have comorbidities such as depression, anxiety, chronic fatigue, insomnia, sexual dysfunction, and fibromyalgia, all of which can impair their quality of life.[2-4] According to a 2013 systematic review, IBS's direct and indirect healthcare costs were estimated to be between $22 and 30 billion.[5] The improved diagnosis of IBS through symptom-based criteria and appropriate testing, along with the lack of reliable biomarkers leading to costly testing and multiple physician visits, has been reported in previous studies.[5-8]

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The Rome IV criteria, which define IBS as abdominal pain with altered bowel habits as the predominant symptom, have recently been introduced as a diagnostic tool.[9] These criteria replaced "discomfort" with "abdominal pain" to enhance specificity and cultural sensitivity. The Bristol Stool Scale is used for subtyping IBS based on stool form and frequency.[10] Despite ongoing research, the underlying pathophysiology of IBS remains unclear, with proposed mechanisms including altered intestinal motility, immune dysfunction, autonomic nervous system dysregulation, changes in the gut microbiome, brain–gut axis alterations, and genetic and psychosocial factors.[2]

Enterochromaffin cells synthesize most of the enteric 5-HT in the gut[9] released in response to mechanical and chemical stimuli.[10] The selective serotonin reuptake transporter (SERT), expressed in all intestinal epithelial cells, is responsible for terminating 5-HT signaling.[11,12] Monoamine oxidase A (MAOA), encoded by the MAOA gene, is the primary enzyme responsible for the degradation of 5-HT in colonic tissues.[13-15] Several studies report decreased SERT expression in the gut of patients with IBS, which may contribute to the increased 5-HT level in their colon. Moreover, SERT null mice exhibit watery diarrhea with enhanced colonic motility and visceral hypersensitivity.[16] The release of 5-HT from enterochromaffin cells is regulated by 5-HT receptors. The activation of 5-HT3 auto receptors triggers a positive feedback mechanism.[17,18] The uptake of 5-HT is facilitated by SERT, a selective serotonin transport protein. Studies have identified altered mucosal 5-HT content and the expression of TPH-1 and SERT in mucosal colonic biopsies of IBS patients. Abnormalities in serine protease and serotonergic signaling pathways might be underlying visceral hypersensitivity in the small intestine. To investigate this. The mRNA expression levels of TPH-1 and SERT content in mucosal colon biopsies were compared between IBS patients and healthy subjects.[19]

Materials and Methods

The subject of the study

A case-control study includes 27 mucosal colonic biopsies obtained from individuals with IBS based on Rome IV and Manning criteria. In comparison, 16 mucosal biopsies were collected from healthy individuals. Additionally, 50 venous blood samples were collected from patients and healthy controls, each from January 2022 until December 2022. The IBS patients were categorized into three subgroups—constipation-predominant IBS (IBS-C), diarrhea-predominant IBS (IBS-D), and mixed-type IBS—based on the Rome IV criteria. Colonoscopy or sigmoidoscopy was used to rule out organic gastrointestinal (GI) disorders. Individuals with certain medical conditions (such as celiac disease, diabetes mellitus, major abdominal surgery, endocrine, central nervous system, or severe psychiatric disorders) were excluded based on history taking, physical examination, laboratory tests, colonic biopsy, lactose tolerance test, and calprotectin test. Healthy subjects were assessed using questions about their medical history and the Rome IV and Manning criteria. During endoscopy, none of the subjects showed abnormality or inflammation in the small intestine. The study was approved by the medical ethics committee of the GI tract center, and all participants provided written informed consent.

Study protocol

Following an overnight fast, the participants underwent a lower GI colonoscopy, during which two mucosal biopsy samples of the sigmoidal colon were collected from each subject. The biopsies were kept with triazole and stored at -80°C for later use.[20]

mRNA expression analysis

The RNeasy Micro Kit from Qiagen extracted total RNA from the collected biopsies. Spectrophotometric quantification of the RNA was measured, and the A260/A280 ratios fell within the normal range. The quality of the RNA was assessed using denaturing agarose gel electrophoresis. Next, first-strand cDNA was synthesized from 1 μg of total RNA. The RNA was extracted from the sample following the TRIzol reagent protocol, eluted in RNAse-free water, and stored at -80°C. The polymerase chain reaction (PCR) amplification was done using the GoTaq 1-Step RT-qPCR system with SYBR Green PCR mix (Promega Corporation, Madison, Wisconsin, USA). Macrogen Company (Macrogen Inc., Seoul, South Korea) synthesized and ordered the primers used. Quantitative real-time RT-PCR was used to analyze the mRNA expression levels of TPH-1 and SERT. The PCR reactions were set up in a 25 μL volume containing 5 μL of diluted cDNA, 12.5 μL of 2× iQ SYBR Green Supermix from Bio-Rad, and 300 nmol/l of both forward and reverse primers. The primers were mRNA/cDNA-specific, designed on intron/exon boundaries or flanking an intron to avoid signal formation from contaminating genomic DNA. A list of the specific primers used is provided in Table 2.[21] The PCR protocol began with a 3-minute denaturation and enzyme-activating step at 95°C. The reaction conditions for amplification are provided in Table 1. Following amplification, a melting curve analysis was performed by gradually increasing the temperature from 55 to 95°C in increments of 0.5°C and measuring fluorescence at each temperature for 10s. All cDNA samples were analyzed in duplicate.

The primer sequences for SERT, as reported in reference,[20] are provided in Table 1.

Immunological test

Measurement of SERT concentration

The kit was a sandwich enzyme immunoassay for the in vitro quantitative measurements of SERT in human samples.

Table 1: Oligonucleotides with a 5′–3′ orientation are used with specific thermal cycling conditions

Gene	Forward primer Reverse primer	PCR product (bp)	Amplification
SERT F	TGGTTCTATGGCATCACTCAGTTC	148	15 s (95 C) 30 s (60 C) 30 s (72°C)
SERT R	GTTGTGGCGGGCTCATCAG		

PCR = polymerase chain reaction

Table 2: The primers for β-actin, used as a housekeeping gene, are reported in reference[20]

β-actin-F	5′-CCCTGGACTTCGAGCAAGAG-3′
β-actin-R	5′-TCACACTTCATGATGGAGTTG-3′

Statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) version 23.0 (SPSS, IBM Company, Chicago, IL, USA) a one-way analysis of variance. An independent samples *t*-test was used, a statistical test that compares the means of two independent groups to determine if there is a significant difference between them. A *P*-value of less than 0.05 was considered significant, which means that there is less than a 5% probability that the observed difference between the two groups was due to chance. The standard deviation of the means was represented by error bars. The data were analyzed using GraphPad Prism 7 and Excel software.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol, the subject information, and the consent form were reviewed and approved by a local ethics committee according to document number B220103 on January 17, 2022 to get this approval.

Results

Serotonin transporter gene expression

Data regarding serotonin expression was found to be nonnormally distributed. Accordingly, they were expressed as median and rand (beside mean ± SD) and analyzed with a nonparametric Mann–Whitney *U* test. The relative median expression of the serotonin transporter gene was 1.05 folds (range 0.12–200 folds) in patients compared to 0.5 folds (range 0.07–12.43 folds) in control with no significant difference [Table 3 and Figure 1].[1]

Serum levels of serotonin transported

The median serum level of the serotonin transporter in IBS patients was 12.8 pg/mL (range 0.6–36.13 pg/mL) with a mean lower than that of controls (median = 11.89 pg/mL, range = 9.44–33.5 pg/mL) with no significant difference [Table 4 and Figure 2].[2]

Table 3: Serot. Transporter gene expression in patients and controls

Variables	Patients ($n = 27$)	Controls ($n = 16$)	*P*-value
Sero. Transporter (fold)			
Mean ± SD	12.81 ± 40.73	1.74 ± 4.02	0.086
Median	1.05	0.55	
Range	0.12–200	0.07–12.43	

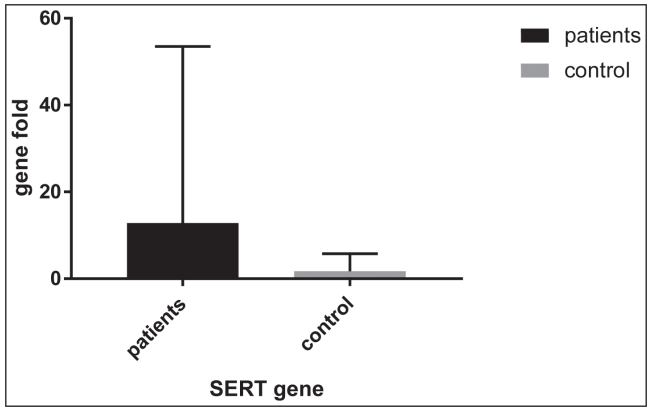

Figure 1: Large intestinal mucosal SERT content increases in IBS patients compared to healthy subjects. All values represent the mean (±SD) of the SERT fold ($P = 0.08$)

Table 4: Serum level of serotonin transporter (SERT) in patients and controls

Variables	Patients ($n = 50$)	Controls ($n = 50$)	*P*-value
Sero. Transporter (pg/mL)			
Mean ± SD	13.68 ± 7.53	15.0 ± 7.08	0.852
Median	12.8	11.89	
Range	0.6–36.13	9.44–33.5	

Discussion

A serotonin reuptake transporter is a protein that regulates the level of serotonin in the body by transporting it from the synapse back into the presynaptic neuron. Research suggests that altered expression of the serotonin transporter gene and changes in serum levels of serotonin may be associated with the development of IBS. Some studies have reported increased serotonin transporter gene expression and higher serotonin levels in the colon tissue and serum of IBS patients compared to healthy controls. However, not all studies have found significant differences, and the relationship between serotonin transporter and

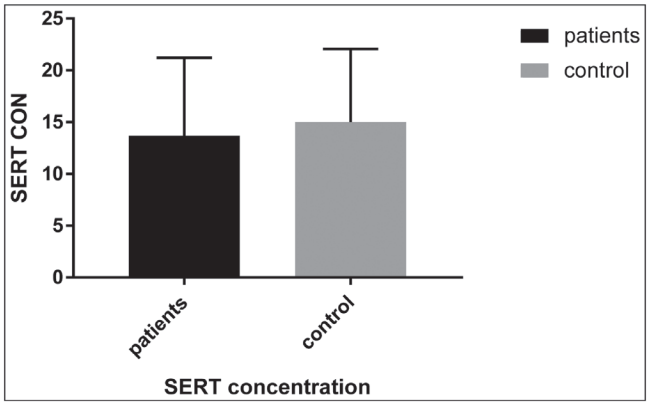

Figure 2: Serum SERT content is increased in control compared to patients

IBS is not fully understood. Further research is needed to determine the precise role of serotonin transporter in IBS and its potential as a therapeutic target.

Several studies have investigated the potential link between serotonin transporter gene expression and serum levels in patients with IBS. For instance, a study by Park *et al.*[22] found that serotonin transporter gene expression was significantly higher in the colon tissue of IBS patients than in healthy controls. Similarly, a study[23] reported that serum levels of serotonin were significantly higher in IBS patients compared to healthy controls. However, not all studies have yielded significant findings. For example, a study by Dinan *et al.*[24] found no significant differences in serotonin transporter gene expression between IBS patients and healthy controls. Additionally, a study by Lee *et al.*[25] found no significant differences in serum levels of serotonin between IBS patients and healthy controls. These discrepancies may be due to various factors, including the subtype of IBS, symptom severity, and differences in measurement techniques. Moreover, other factors such as stress and diet may impact serotonin levels and potentially contribute to IBS symptoms.

Another study suggests that mRNA expression of TPH-1 and SERT in the colon is increased in IBS patients compared to controls. The authors concluded that discrepancies in previous studies on serotonergic pathway components in IBS patients may be due to differences in the portion of IBS patients exhibiting colorectal hypersensitivity. The findings are consistent with a previous study by Coates *et al.*[26,27] reported reduced TPH-1 and SERT mRNA expression in the bowel of IBS patients but inconsistent with a study by Camilleri *et al.*, which did not find a significant difference in SERT mRNA expression.

Conclusion

According to the current study's findings, high SERT gene expression and its serum level related to visceral hypersensitivity lead to worsened development of IBS symptoms.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Canavan C, West J, Card T. The epidemiology of irritable bowel syndrome. Clin Epidemiol 2014;6:71-80.
2. Sibelli A, Chalder T, Everitt H, Taylor J, Jackson D, Smith R, *et al*. The role of cognitive behavioral therapy for patients with irritable bowel syndrome: A systematic review. J Psychosom Res 2016;91:10-23.
3. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: A meta-analysis. Clin Gastroenterol Hepatol 2012;10:712-721.e4.
4. Mohammed BI, Amin BK. Sociodemographic characteristics, smoking, and family history of patients with inflammatory bowel disease, northern part of Iraq. Med J Babylon 2022;19:615-9.
5. Lacy BE, Mearin F, Chang L, Müller-Lissner S, Ford AC, van Oudenhove L, *et al*. Bowel disorders. Gastroenterology 2016;150:1393-1407.e5.
6. Cash BD, Chey WD. Irritable bowel syndrome—An update on diagnosis and treatment. JAMA 2015;313:949-58.
7. Houghton LA, Heitkemper M, Crowell M, Sullivan S, Jones S, Mitchell S, *et al*. Age, gender and women's health and the patient. In: Drossman DA, Corazziari E, Delvaux M, *et al*., editors. Rome III: The Functional Gastrointestinal Disorders. 3rd ed. McLean, VA: Degnon Associates, Inc.; 2006. p. 441-72.
8. Agrawal A, Whorwell PJ. Review article: Abdominal bloating and distension in functional gastrointestinal disorders—Epidemiology and exploration of possible mechanisms. Aliment Pharmacol Ther 2008;27:2-10.
9. Lacy BE, Mearin F, Chang L, Ford AC, Drossman DA, van Oudenhove L, *et al*. Bowel disorders. Gastroenterology 2016;150:1393-1407.e5.
10. Raybould HE. Mechanisms of CCK signaling from gut to brain. Curr Opin Pharmacol 2007;7:570-4.
11. Bertrand PP, Thomas EA, Marciani L, Morris GP, Bornstein JC. Involvement of 5-HT3 receptors in the mechanism of action of the antidepressant fluoxetine in neuropathic pain and spinal cord injury. Neuropharmacology 2008;55:983-8.
12. Gershon MD. Review article: Serotonin receptors and transporters—Roles in normal and abnormal gastrointestinal motility. Aliment Pharmacol Ther 2004;20:3-14.
13. Wade PR, Chen J, Jaffe B, Kassem IS, Blakely RD. The distribution and regulation of the serotonin transporter in the gastrointestinal tract. Am J Physiol Gastrointest Liver Physiol 1997;272:G601-10.
14. Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H, *et al*. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. Gastroenterology 2004;126:1657-64.
15. Sjölund K, Ekman R, Lindgren S, Rehfeld JF, Sundler F. Altered expression of serotonin receptor subtypes in colon in patients with irritable bowel syndrome and ulcerative colitis. Neurogastroenterol Motil 2006;18:200-9.
16. Neumann D, Seifert R. The therapeutic potential of histamine receptor ligands in inflammatory bowel disease. Biochem Pharmacol 2014;91:12-7.
17. Chen JJ, Li Z, Pan H, Murphy DL, Tamir H, Koepsell H, *et al*. Maintenance of serotonin in the intestinal mucosa and ganglia of mice that lack the high-affinity serotonin transporter: Abnormal

intestinal motility and the expression of cation transporters. J Neurosci 2001;21:6348-61.
18. Gebauer A, Merger M, Kilbinger H. Modulation by 5-HT3and 5-HT4 receptors of the release of 5-hydroxytryptamine from the guinea-pig small intestine. Naunyn Schmiedebergs Arch Pharmacol 1993;347:137-40.
19. Racke K, Reimann A, Schworer H, Kilbinger H. Regulation of 5-HT release from enterochromaffin cells. Behav Brain Res 1996;73:83-7.
20. Farhan SS, Tahmasebi P, Al-Dahmoshi HO, Gatea HS. Gene expression of TNF-α among Iraqi COVID-19 patients with a different severity status. Med J Babylon 2023;20:90-4.
21. Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H, *et al*. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. Gastroenterology 2004;126:1657-64.
22. Kerckhoffs AP, Ter Linde JJ, Akkermans LM, Samsom M. Trypsinogen IV, serotonin transporter transcript levels and serotonin content are increased in small intestine of irritable bowel syndrome patients. Gastroenterology 2008;135:781-9.
23. Park JH, Rhee PL, Kim G, Lee JH, Kim YH, Kim JJ. Increased expression of serotonin transporter in the colon of patients with irritable bowel syndrome. Ann Surg Treat Res 2013;84:250-4.
24. Park JH, Rhee PL, Kim G, Lee JH, Kim YH, Kim JJ. Serum serotonin levels are not altered in patients with irritable bowel syndrome with diarrhea: A pilot study. Korean J Intern Med 2016;31:1147-54.
25. Dinan TG, Quigley EM, Ahmed SM, Scully P, O'Brien S, O'Mahony L, *et al*. Hypothalamic–pituitary–gut axis dysregulation in irritable bowel syndrome: Plasma cytokines as a potential biomarker? Gastroenterology 2006;130:304-11.
26. Lee JH, Park JH, Kim YH, Kim JH, Rhee PL. Serum serotonin level is not increased in irritable bowel syndrome patients compared with healthy controls. J Neurogastroenterol Motil 2017;23:221-5.
27. Sarrouilhe D, Dejean C, Mesnil M. Inhibitors of serotonin reuptake and their role in the treatment of irritable bowel syndrome. Clin Exp Gastroenterol 2019;12:29-39.