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Comparison of the Effectiveness of Hypnotherapy and Cognitive-Behavioral Therapy on Chronic Pain Indices and Cognitive-Emotional Regulation in Patients with Irritable Bowel Syndrome

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Abstract

Background: There is evidence that irritable bowel syndrome (IBS) can be a chronic and prevalent condition that is more common in individuals with psychological disorders than in the general population.
Objectives: This study aimed to compare the effectiveness of hypnotherapy and cognitive-behavioral therapy (CBT) in mitigating chronic pain and cognitive-emotional regulation in patients with IBS.
Methods: In this three-arm randomized clinical trial, participants who were adults with refractory IBS were screened. Co-primary outcomes were chronic pain indices and cognitive emotion regulation at a six-month follow-up. The statistical population of this study was all patients with IBS referred to Masoud Clinic and Shariati Hospital from May 2019 to February 2021 in Tehran, Iran. Twenty-four patients were calculated for each group, and 72 were for two experimental and one control group using convenience sampling. Seventy-two patients with IBS were accessible to us after the inclusion and exclusion criteria. They were selected and randomly assigned to either interventions or the control group (n = 24). The demographic checklist, Chronic Pain Grade Questionnaire (CPGQ), and Cognitive Emotion Regulation Questionnaire (CERQ) were used in three periods.
Results: Data were analyzed by repeated-measures analysis of variance. Preliminary findings showed that the effectiveness of both treatments on chronic pain indices and cognitive emotion regulation in the post-test stage was significant ($P < 0.05$). Secondary results showed that treatment efficacy remained stable until the follow-up stage.
Conclusions: This study revealed hypnotherapy and cognitive-behavioral therapy could effectively treat patients with irritable bowel syndrome. Patients with IBS could benefit from psychological intervention based on these findings.

Keywords: Chronic Pain, Cognitive Behavioral Therapy, Emotional Regulation, Hypnotherapy

1. Background

Irritable bowel syndrome (IBS) symptoms include constipation, diarrhea, and abdominal pain. The diagnosis of somatic symptom disorder occurs when there are positive signs and symptoms without an explanation from a physician. To meet the ROME III criteria, a patient with IBS must also experience recurrent abdominal pain at least one day each week for the past three months. Possible symptoms include abdominal pain relieved by defecation, abdominal pain with increased stool, and abdominal pain with deformed stool (1).

The cause of IBS symptoms is currently the subject of several theories. According to the most widely accepted theory, gut motility is dysregulated, visceral sensitivity is heightened, and psychological factors are involved (2). In more severely affected patients, psychological factors significantly contribute to symptom onset, expression, and disorder progression. In patients with IBS, situations that are accompanied by excessive negative emotions, social isolation, the inability to control and manage anxiety, and feelings of helplessness can be exacerbated. As a result, it can be concluded that there is a relationship between symptoms of emotional regulation and IBS severity.

Several other strategies, such as cognitive emotion regulation strategies, have also been proposed by Garnefski et al. (3). A synonym for coping strategy is a method for managing and controlling emotionally evoked input information. In particular, this is true when a person has been exposed to a negative emotional experience or a threatening situation. There are nine methods for regulating cognitive emotions or coping with life stresses. The strategies include self-esteem, ruminating, catastrophizing, perspective, and acceptance. Ruminating, self-harming, and catastrophizing can also lead to psychological distress (4).

There was a correlation between the quality of life and chronic constipation; and a decrease in quality of life was observed in this disorder (5). Several studies have shown that modifying or changing multiple emotional states is impossible without using mature emotion regulation strategies (6, 7). It is essential to have high cognitive processes such as willpower, purposeful thinking, planning, attention, decision-making, self-organization, self-discipline, and self-discipline mindfulness to succeed in life, learn tasks, and take intelligent actions. Consequently, it is necessary to pay attention to regulating patients' emotions to assist them in managing them. For this reason, there is a need for specific psychological interventions. Patients unable to use medication can be treated for IBS without gold-standard treatment (8).

Using hypnosis as a therapeutic tool involves both induction and induction of visual experiences. Hypnosis aims to induce a change of consciousness in the subject. There are indications of changes in mental expertise, perception, emotion, excitement, thinking, or behavior (9). The state of mind in which one is thoroughly distracted from one subject and focuses entirely on another. Hypnotherapy is labor-intensive, time-consuming, as well as extremely expensive. This medication should only be used in cases of severe IBS. A mild patient is unlikely to benefit from the technique. This is because the case does not exhibit the motivation and dedication necessary for the method to be effective.

In addition to reducing the overall cost, non-medical individuals with psychosocial or biomedical training can provide these services. In a hospital setting, a group of therapists can bring several benefits. This enhances the legitimacy and credibility of the treatment. In addition, therapists can share their experiences, and a healthcare expert can monitor the treatment process. Medical supervision allows therapists to receive advice and discuss relevant issues on an ongoing basis. The patient may report previously undisclosed symptoms of significance due to a more prolonged relationship with the therapist, which may require further investigation. It has been demonstrated in a systematic review of 800 patients with IBS that hypnotherapy interventions have long-term effects on their symptoms. It showed the effect of hypnotherapy on the management and control of symptoms in patients with IBS and that it could effectively control and manage the symptoms of IBS (10, 11). Previous work has also demonstrated that hypnotherapy treatment may affect the perception of experimental visceral stimuli in hypersensitive and hyposensitive patients. However, changes in the perception of rectal distension did not correlate with symptom improvement in pediatric or adult patients. Despite the successful use of hypnotherapy for treating IBS symptoms, the neurobiological mechanisms underlying this mind-based therapy remain unknown.

Psychological interventions such as cognitive behavior therapy (CBT) and hypnosis effectively treat IBS (12). It was shown that cognitive-behavioral psychotherapy and drug therapy had different results in treating IBS than medication (13). An evaluation of cognitive-behavioral techniques for training coping strategies confirmed the effectiveness of this method in reducing the symptoms of IBS. It is estimated that three patients will require treatment (14). Several international guidelines recommend CBT as a treatment for IBS (15). In cognitive-behavioral interventions, individuals are taught how to test their beliefs and thoughts as hypotheses for validity. Using this method, cognitive reconstruction techniques and coping strategies are developed to overcome dysfunctional thoughts, negative emotions, and stress. As a result of CBT, distress levels are influenced by a patient's lifestyle, behavioral patterns, and attitudes towards themselves and their environment. The assessment process will determine which cognitive and behavioral patterns are perpetuating or exacerbating the symptoms of IBS. Until now, few controlled clinical trials have examined the effectiveness of such programs in treating IBS.

There are many similarities between cognitive behavioral therapy and hypnosis when it comes to treating gastrointestinal (GI) disorders. It is important for the patient to practice at home between sessions with the therapist to prepare for the next session. It is a brief and time-limited therapy. A substantial body of research supports both of these interventions. IBS patients generally benefit from both interventions.

2. Objectives

These two therapies are different: hypnotherapy (mind-body intervention) and the other CBT (psychosocial intervention) have been used in many fields to help different patients. Therefore, the current study aimed to compare hypnotherapy and CBT on chronic pain and emotional regulation in patients with IBS.

3. Methods

Adults with refractory IBS (symptoms persisting for at least 12 months, despite using first-line treatment) were di-

Table 1. Hypnotherapy Intervention Protocol

Session	Content
Session 1	Detailed clinical history of the patient to diagnose and identify important environmental, social, biological, and psychological factors of his/her behaviors, setting realistic goals and expectations of treatment, initial explanation of treatment method
Session 2	They are performing hypnosis, deepening and indoctrination of relaxation (pain control), especially the entire abdomen and intestines, and post-hypnotic conditioning-training and performing similar self-hypnosis exercises at home
Session 3	Strengthen the ego and do similar self-hypnosis exercises at home
Session 4	Relaxation controlling room
Session 5	Intestinal concentrated exercises in trance (Mental imaging-controlling and normalizing movements and function) performing similar self-hypnosis exercises at home
Session 6	Age regression and coping with past stressful life events in a trance and doing similar self-hypnosis exercises at home
Session 7	Time progress and going to time without any problems in the future in a trance and doing similar self-hypnosis exercises at home
Session 8	Summarize the previous sessions and emphasize continuing medication and self-hypnosis exercises

vided into three arms of the randomized trial. Co-primary outcomes were chronic pain indices and cognitive emotion regulation at a two-month follow-up. The statistical population of this study was all patients with IBS referred to Masoud Clinic and Shariati Hospital from May 2019 to February 2021 in Tehran, Iran. Twenty-four patients were calculated for each group, and 72 were for two experimental and one control group using convenience sampling. Seventy-two patients with IBS were accessible to us after the inclusion and exclusion criteria. They were selected and randomly assigned to either interventions or the control group (n = 24).

Inclusion criteria were as follows: a gastroenterologist had to diagnose IBS according to ROME III diagnostic criteria, receiving drug treatment under the supervision of a specialist, not receiving psychological treatment within the past three months, having at least a diploma, and being between the ages of 20 and 50. Exclusion criteria included traveling from different parts of the city, receiving psychological treatment for the past six months, and being absent more than twice during intervention sessions.

3.1. Interventions' Content

Hypnotherapy: This therapy was developed for the first time in Iran, and it was prepared based on general modeling of all available references (13). The treatment sessions included eight sessions of individual therapy once a week for one and a half hours. Homework and self-hypnosis were presented to the patients (Table 1).

3.2. Cognitive-Behavioral Therapy

This therapy was based on the general modeling of the total available resources and treatment package of Zomorodi et al., (14). Iranian patients with irritable bowel syndrome were treated with behavioral and educational methods adapted to their conditions and cultures. Eight sessions of 1.30 hours were conducted once a week for psychosomatic disorders (Table 2).

3.3. Measures

3.3.1. Demographic Checklist

This checklist was prepared and used by the researcher to assess the demographic characteristics of study participants, such as age, sex, level of education, and duration of illness.

3.3.2. Rome III Diagnostic Criteria

Gastroenterologists use it to diagnose IBS. This diagnostic criterion was introduced to the scientific community in 2006 under the name of Rome III, which has a high standard, after various corrections made with the cooperation of many digestive experts worldwide (15).

3.3.3. The Irritable Bowel Syndrome Severity Scale

Five items on the Irritable Bowel Syndrome Severity Scale (IBSS) are rated on a 100-point scale: severity of abdominal pain, frequency of abdominal pain, abdominal distension, dissatisfaction with bowel habits, and interference with quality of life (16). Each component contributes equally to the final score, with a higher score indicating a more severe condition.

3.3.4. The Chronic Pain Grade Questionnaire

Using the Chronic Pain Grade Questionnaire (CPGQ), chronic pain severity is measured on three dimensions: persistence, intensity, and disability. Based on the results of the questionnaire, patients are divided into five hierarchical categories. Based on the responses to seven items, a subscale of the CPGQ is calculated. There are three grades of disability: low disability and low intensity (Grade I), high disability and moderately limiting intensity (Grade III), and severe disability and severely limiting intensity (Grade IV) (17).

Table 2. CBT Intervention Protocol

Session	Content
Session 1	Detailed clinical history of the patient to diagnose and identify important environmental, social, biological, and psychological factors of his/her behaviors, setting realistic goals and expectations of treatment method, initial explanation of treatment, training in relaxation techniques, abdominal breathing, homework and familiarity with the form of weekly activities
Session 2	Reviewing the weekly activity form and helping to reinforce positive behaviors, and monitoring clients' relaxation training
Session 3	Helping to reinforce positive behaviors in the weekly activity form and teaching the cognitive pattern, providing negative automatic thoughts recording sheets-Identifying thoughts that provoke symptoms and unpleasant feelings. Investigating possible problems in recording thoughts and identifying emotions, and helping to solve them.
Sessions 4, 5, 6, and 7	Evaluation of the form of negative automatic thoughts and teaching how to evaluate thoughts (verbal challenge and behavioral testing), using methods such as down-arrow, analysis of advantages and disadvantages, and Socratic questioning of beliefs that create unpleasant feelings. Provide a progressive muscle relaxation training tape and encourage the person to do so.
Session 8	Summarizing the contents of the last seven sessions. Paying attention to patients' intermediate beliefs and underlying assumptions. Getting feedback from patients on treatment sessions

3.3.5. The Cognitive Emotion Regulation Questionnaire

It is a self-report tool that was developed in 1999 by Garnefski & Kraaij (18). A 5-point Likert scale is used to grade 18 items. The Cognitive Emotion Regulation Questionnaire (CERQ) measures cognitive emotion regulation strategies in response to life-threatening and stressful events based on nine subscales: self-blame, other blame, ruminating, catastrophizing, putting things in perspective, refocusing, reappraising, accepting, and refocusing on planning. A higher score indicates greater use of the strategy in each subscale.

3.4. Statistical Analysis

We used SPSS-25 software to analyze the data using repeated measures analysis of variance. Our study compared the effectiveness of hypnotherapy with Cognitive Behavioral Therapy (CBT) based on longitudinal multilevel modeling (LMLM). A Shapiro-Wilk test was conducted to determine whether all data were standard. Shapiro-Wilk tests are usually conducted for small samples to determine the normality of the data.

4. Results

The results are presented in three sections. (I) reports on the characteristics of the sample, as well as the PSM analysis, and (II) reports the results of the LMLM analysis. The effect sizes and dropout rates for the two therapies and a control group are summarized in section III. In this study, 113 (64%) eligible patients were randomly assigned to the first step of the study: 67% women, 33% men, and a mean age of 37.13 years. Seventy-five percent of the patients completed their follow-up after two months.

In this study, 72 patients with IBS were randomly divided into three groups of 24 participants. Four people in the hypnotherapy group, three people in the CBT group, and four people in the control group were excluded from the study during treatment. In the end, 20 people remained in the first group, 21 in the second group, and 20 in the control group. The study involved 61 participants, of which 64% were women and 36% were men. Among the participants, the mean age was 35.34 years, and the standard deviation (SD) was 7.33 years. Demographic characteristics of the experimental and control groups are provided in Table 3. Also, no significant differences were found between research groups regarding gender, age, marriage, education, duration of illness, and smoking (Table 3).

Chronic pain showed a significant difference between the three groups' mean scores of pre-test, post-test, and follow-up (Table 4). The effects of intra-subjects for adapted cognitive emotion regulation showed a significant difference between the mean scores of pre-tests, post-test, and follow-up in the three groups (Table 5). Also, the effects of intra-subjects for un-adapted cognitive emotion regulation showed a significant difference between the mean scores of pre-tests, post-test, and follow-up in the three groups (Table 6).

Comparison of adjusted means shows that two experimental groups are significantly influential on chronic pain, adapted and un-adapted cognitive emotion regulation in patients with IBS, and hypnotherapy compared to CBT with an effect size of 0.34 in reducing chronic pain, 0.29 decrease in un-adapted cognitive emotion regulation and with an effect size of 0.13 is effective in increasing adapted cognitive emotion regulation. Also, the lack of significant differences in comparing follow-up scores versus post-test means that changes in experimental groups over time have a lasting effect.

5. Discussion

This study has shown that hypnotherapy and cognitive-behavior therapy significantly decreased chronic pain symptoms and improved emotional regulation; moreover, their effects were stable after a six-month follow-up.

Table 3. Demographic Characteristics of People with IBS Characteristics [a]

Characteristics	HT	CBT	Control	Total	P-Value
Gender					0.899
Female	12 (60)	14 (67)	13 (65)	39 (64)	
Male	8 (40)	7 (33)	7 (35)	22 (36)	
Age					0.053
20 - 29	8 (40)	2 (9.5)	3 (15)	13 (21)	
30 - 39	8 (40)	14 (67)	7 (35)	29 (48)	
40 - 49	4 (20)	5 (23.5)	10 (50)	19 (31)	
Marriage status					0.950
Single	9 (45)	9 (43)	8 (40)	26 (43)	
Married	11 (55)	12 (57)	12 (60)	35 (57)	
Education					0.701
Diploma	2 (10)	6 (29)	7 (35)	15 (24)	
Associate	2 (10)	2 (9.5)	2 (10)	6 (10)	
BSc	7 (35)	8 (38)	5 (25)	20 (33)	
MSc	6 (30)	4 (19)	5 (25)	15 (24)	
PhD	3 (15)	1 (4.5)	1 (5)	5 (9)	
Duration of illness					0.899
> 5	7 (35)	8 (38)	5 (25)	20 (33)	
5 - 10	8 (40)	10 (48)	7 (35)	25 (41)	
10 <	5 (15)	3 (14)	8 (40)	16 (26)	
Smoking					0.323
Yes	4 (20)	4 (19)	1 (5)	9 (35)	
No	16 (80)	17 (81)	19 (95)	52 (85)	
Comorbid diseases					0.674
Yes	6 (30)	9 (43)	8 (40)	23 (38)	
No	14 (70)	12 (57)	12 (60)	38 (62)	

[a] Values are expressed as No. (%).

Table 4. Descriptive Indicators of Pre-test, Post-test, and Follow-up of Three Groups in Chronic Pain and Cognitive Emotion Regulation [a]

Variables	Pre-test	Post-test	Follow-up
Experiment 1 (n = 20) Hypnotherapy			
Chronic pain	33.6 ± 13.08	12.65 ± 10.51	14.7 ± 11.09
Adapted cognitive emotion regulation	32.65 ± 6.16	37.75 ± 7.04	34.8 ± 6.96
Un-adapted cognitive emotion regulation	23.85 ± 5.76	19 ± 4.38	19.7 ± 4.41
Experiment 2 (n = 21) CBT			
Chronic pain	32.76 ± 13.01	17.04 ± 15.94	17 ± 14.32
Adapted cognitive emotion regulation	31.47 ± 7.13	34.42 ± 8.82	33.95 ± 9.08
Un-adapted cognitive emotion regulation	26.90 ± 5.55	22.47 ± 6.87	22.95 ± 5.94
Control (n = 20)			
Severity and physical symptoms	310.00 ± 79.63	356.25 ± 72.03	355.00 ± 71.91
Chronic pain	32.15 ± 15.56	38.15 ± 15.55	37.95 ± 14.39
Adapted cognitive emotion regulation	31.65 ± 7.51	30.3 ± 8.32	28.55 ± 7.66
Un-adapted cognitive emotion regulation	23.7 ± 6.34	26.75 ± 6.25	27.9 ± 5.55

[a] Values are expressed as mean ± SD.

Table 5. Findings of Analysis of Variance Test with Repeated Measures of Chronic Pain Variables and Cognitive Emotion Regulation of Three Experimental and Control Groups

Variable Source of Change	Time Stages			Group			Interaction of Stages Group		
	F	P-Value	Eta	F	P-Value	Eta	F	P-Value	Eta
Chronic pain	27.536	0.0001	0.322	10.357	0.0001	0.263	17.768	0.0001	0.380
Adapted cognitive emotion regulation	4.071	0.028	0.066	3.698	0.008	0.115	3.613	0.014	0.111
Un-adapted cognitive emotion regulation	5.044	0.011	0.080	5.873	0.005	0.168	10.746	0.0001	0.270

Table 6. Bonferroni Post Hoc Test for Chronic Pain and Cognitive Emotion Regulation Variables in Research Groups

Variables	I	J	Mean Difference Between I-J	Standard Error	P-Value
Chronic pain					
	Pre-test	Post-test	10.221	1.804	0.0001
		Follow-up	9.621	1.748	0.0001
	Post-test	Follow-up	-0.601	0.925	1.000
	Hypnotherapy	CBT	-1.953	3.743	1.000
Adapted cognitive emotion regulation					
	Pre-test	Post-test	2.234	0.961	0.017
		Follow-up	2.725	0.599	0.011
	Post-test	Follow-up	0.509	0.860	1.0000
	Hypnotherapy	CBT	1.781	2.111	1.000
Un-adapted cognitive emotion regulation					
	Pre-test	Post-test	2.076	0.713	0.015
		Follow-up	2.301	0.729	0.007
	Post-test	Follow-up	-0.775	0.518	0.420
	Hypnotherapy	CBT	-3.261	1.533	0.113

These results align with the study of Everitt et al. (19), which showed that hypnotherapy and cognitive-behavior therapy could considerably improve GI symptoms and psychological well-being of patients with GI problems. Therefore, when hypnotherapy and cognitive behavioral therapy improve individuals' psychological well-being, emotion regulation strategy plays a significant role in the quality of life and psychological well-being.

By altering thoughts, feelings, the patient's subjective assessment of physical symptoms, and reactivity to those symptoms holistically, both hypnotherapy and cognitive-behavioral therapy are effective treatments for IBS. The results of balloon inflation tests have shown normal cortical responses to visceral stimulation (20). According to randomized controlled trials, both of these major brain-gut psychotherapies have shown broadly similar effects, at least in terms of their effectiveness, when applied to similar GI disorders.

Both interventions outperformed various controls. Among the treatment options are medical care, education, waiting lists, placebo pills, antidepressants, and antispasmodics. The majority of patients improved on their primary outcomes despite differences between these therapies. Considering the number of studies done on refractory patients, this is impressive. Both treatment benefits can be maintained without further intervention for six to twelve months after an IBS treatment. The effects of therapy lasted 18 months in several studies (21) and two years in another study (19).

The results of the present study also showed that hypnotherapy in the follow-up stage has a lasting effect on reducing the symptoms of patients with irritable bowel syndrome, which is consistent with Lindfors et al. (22) and inconsistent with Gonsalkorale (13). Socratic dialogue is used in CBT to restructure catastrophic appraisals of gut sensations and to promote a non-reactive attitude toward gut-related thoughts and feelings. While clinical hypnosis utilizes suggestions and imagery to induce relaxation and alter the perception of visceral sensations, CBT does not aim to alter gut trends. Instead, it promotes awareness and

acceptance of such feelings. CBT for IBS may have several therapeutic mechanisms, given these procedural differences, including increasing non-reactivity to gut-focused anxiety and catastrophic thoughts about the ability to manage pain, improving awareness of IBS symptoms as innocuous interceptive signals rather than threats to well-being, reducing psychophysiological stress responses, and facilitating attentional disengagement from gut sensations and obsessive thoughts regarding visceral function. In this study, we add a specific psychological intervention option to clinical interventions for treating chronic irritable bowel syndrome. Furthermore, since CBT can be conducted in moderately large groups, the intervention tested in our study may be more cost-effective than other psychological treatments for IBS.

Psychological interventions for IBS are typically conducted as individual therapy sessions as part of CBT and similar programs. These programs are staffed by experienced instructors, allowing physicians to prescribe standard treatments to their patients while attending these programs. It is safe, compatible with conventional care approaches, and gives patients increased flexibility in managing their symptoms.

The range of pains that can be well treated by hypnosis is extensive, and various studies have shown this method's usefulness in treating various acute and chronic pains. According to the intestinal-oriented hypnotherapy model, indoctrination is provided to control and normalize GI function; metaphors are also used to achieve this goal. During hypnotherapy, patients are directed to a specific state of mind in which they are highly focused and absorbed, followed by deep relaxation and gut-focused imaging and cues to control symptoms and normalize bowel function (23). Research has shown that hypnotherapy improves emotion regulation by affecting some mediating components. Hypnotherapy is effective in enhancing mindfulness and, through this, has been able to improve self-consciousness and play a role in reducing emotional dysregulation. Regarding the effect of hypnotherapy in improving emotion regulation, it can be said that hypnotherapy helps people focus non-judgmentally on how to take care of themselves in daily life, reduce muscle tension, refocus on positive experiences, and manage negative experiences. The output of such interventions is spatial formation in which cognitive fault occurs well, and the individual will not engage in cognitive fusion and emotional dysregulation (24). Social, economic, physical, and interpersonal consequences of IBS include stress, mental instability, and disability. A crisis is inevitable in the context of cognitive behavioral therapy. In spite of unpleasant situations, they can increase their emotions by changing their thinking, attitudes, and behavior. The research results show that people with different emotional regulations adopt different reasoning and perspectives in the face of adverse conditions.

5.1. Limitations

There are limitations to this study. As a result of the convenience sampling method, age regression can be considered an uncontrolled variable in the present study. Since this study did not provide information regarding the severity of the diet, interventions were analyzed without taking into account the severity of the diet. This issue should be considered in future research. Another limitation is the inability to control intervening variables, such as economic status, employment, education, and income. To provide clear insights into the effectiveness of psychological interventions in patients with IBS, future studies should focus on identifying the mechanisms responsible for the effectiveness of psychotherapy. A future goal of clinical studies will be to develop effective and efficient therapies for improvement of GI function.

5.2. Conclusions

It has been shown that psychological interventions, particularly cognitive behavioral therapy, and hypnotherapy, can effectively reduce the frequency and severity of symptoms in patients with refractory IBS symptoms. In patients with abdominal pain and composite primary IBS symptoms, CBT and hypnotherapy may be able to help them. There is no doubt that these interventions may significantly affect the clinical outcome of patients with hard-to-treat IBS.

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Footnotes

Authors' Contribution: Study concept and design: A.P and Z.P.; Analysis and interpretation of data: A.P. and M.P.; Drafting of the manuscript: A.S and H.P; Critical revision of the manuscript for important intellectual content: Z.P, M.P, and A.P; Statistical analysis: A.P.

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