

The Efficacy of the Kampo Formula Keishikashakuyakuto for Irritable Bowel Syndrome: A Phase 3, Multicenter, Double-Blind, Placebo-Controlled, Randomized Controlled Trial

Takuma Higurashi¹, Akiko Fuyuki¹, Hidenori Ohkubo¹, Hiroshi Iida¹, Masahiko Inamori¹, Masataka Taguri², Yasuhiko Komiya³, Shungo Goto⁴, Leo Taniguchi⁴, Naoya Okada⁵, Takafumi Ito⁵, Akira Mizuki⁵, Noriaki Manabe⁶, Ken Haruma⁶, Mitsuo Nagasaka⁷, Yoshihito Nakagawa⁷, Naoki Ohmiya⁷, Sayuri Yamamoto⁸, Yasushi Funaki⁸, Kunio Kasugai⁸ and Atsushi Nakajima¹*

¹Department of Gastroenterology and Hepatology, Yokohama City University School of Medicine, Yokohama, Japan

²Department of Biostatistics, Yokohama City University School of Medicine, Yokohama, Japan

³Department of Gastroenterology, Yokosuka General Hospital, Uwamachi, Japan

⁴Department of Gastroenterology, Hiratsuka City Hospital, Kanagawa, Japan

⁵Department of Gastroenterology, Keiyu Hospital, Kanagawa, Japan

⁶Division of Endoscopy and Ultrasonography, Department of Clinical Pathology and Laboratory Medicine, Kawasaki Medical School, Kurashiki, Japan

⁷Department of Gastroenterology, Fujita Health University School of Medicine, Aichi, Japan

⁸Department of Gastroenterology, Division of Internal Medicine, Aichi Medical University School of Medicine, Aichi, Japan

*Corresponding author: Atsushi Nakajima, Department of Gastroenterology and Hepatology, Yokohama City University School of Medicine, 3-9 Fuku-ura, Kanazawa-ku, Yokohama 236-0004, Japan, Tel: +81-45-787-2640; Fax: +81-45-784-3546; E-mail: nakajima-ky@umin.ac.jp

Received date: May 08, 2017; Accepted date: May 14, 2018; Published date: May 16, 2018

Copyright: ©2018 Higurashi T, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Background: Irritable bowel syndrome (IBS) is a common gastrointestinal functional disorder characterized by abdominal pain and altered bowel habits in the absence of any structural abnormality. IBS patients often suffer from abdominal symptoms and severe reduction in quality of life (QOL). The Kampo formula keishikashakuyakuto (KST) is considered effective for IBS abdominal pain. However, there are few high-quality randomized controlled trials of Kampo treatment of IBS.

Methods: This will be a multicenter, double-blind, placebo-controlled, randomized controlled trial using patients who fulfil the Rome IV criteria for IBS. All eligible patients will be randomly allocated into either a KST group or a placebo group. Patients in the KST group will receive an oral dose of 2.5 g KST three times per day before or between meals for 8 weeks. Patients in the placebo group will receive placebo medicine with the same frequency as the KST group. IBS-QOL and IBS severity index scores for the two groups will be compared before and after treatment.

Discussion: This will be the first study to assess the effect of KST on QOL in IBS patients. Kampo medicine is believed to improve IBS-associated symptoms; however, its mechanism of efficacy is still unknown. Clear evidence that KST is effective for IBS would expand the therapeutic options for the disease and have a substantial clinical impact.

Registration: This trial has been registered in the University Hospital Medical Information Network Clinical Trials Registry as UMIN000026235.

Funding: Research for creating scientific knowledge about Kampo medicine from the Japan Agency for Medical Research and Development.

Keywords: Irritable bowel syndrome; Kampo medicine; Keishikashakuyakuto; Randomized controlled trial; IBS-QOL

Abbreviations: IBS: Irritable Bowel Syndrome; QOL: Quality Of Life; KST: Keishikashakuyakuto; CAM: Complementary and Alternative Medicine; IBS-D: IBS with diarrhoea; IBS-C: IBS with Constipation; IBS-M: IBS with mixed Bowel patterns of Constipation and Diarrhoea; IBS-U: IBS with unspecified Patterns; IBS-SI: IBS-Severity Index; SD: Standard Deviation

Introduction

Irritable bowel syndrome (IBS) is a common gastrointestinal functional disorder characterized by abdominal pain and altered bowel habits in the absence of any structural abnormality [1]. IBS has an estimated incidence of 10-22% worldwide, making it the most prevalent gastrointestinal functional disorder [2,3]. Based on the Rome IV criteria, IBS has been classified into subtypes according to the predominant stool pattern, such as IBS with diarrhea (IBS-D), IBS with constipation (IBS-C), IBS with mixed bowel patterns of constipation and diarrhea (IBS-M), and IBS with unspecified patterns (IBS-U) [4].

IBS patients often suffer from abdominal symptoms and their quality of life (QOL) is severely affected; this interferes with their working ability, daily activity, and psychological status. Furthermore, as IBS is more commonly diagnosed in patients younger than 50 years [5], the disease leads to substantial economic loss through decreased work productivity and increased health care costs [6]. Moreover (and most importantly), most patients are dissatisfied with their current treatments [7]. Therefore, effective management of IBS is very important. However, there are no definitive treatments for IBS, because it is characterized by diverse symptomatology and a complicated pathogenesis. Currently, IBS patients are offered various treatments according to their symptoms; these include diet therapy, behavior therapy, antibiotics, purgative drugs, antidiarrheal drugs, antidepressants, and Kampo medicine. Kampo medicine (i.e., traditional Japanese medicine) was introduced into Japan from China via the Korean Peninsula 1500 years ago. This treatment approach has been shaped over a relatively long period of time into a unique Japanese medicine by the Japanese climate and culture [8]. Kampo medicine is now a completely different system from traditional Chinese and Korean medicine. Complementary and alternative medicine (CAM) currently attracts global attention. Among doctors in Japan, Kampo medicine is the most trusted and most frequently practiced of the CAM therapies [9,10]. Kampo medicine is considered effective for IBS-associated symptoms. Sasaki et al. reported that the Kampo formula keishikashakuyakuto (KST) is effective at improving abdominal pain in IBS patients. KST contains various natural ingredients, such as shakuyaku (*Paeonia lactiflora* Pall), keihi (*Cinnamomum cassia* Blume), taiso (*Zizyphus vulgaris* Lamarck var. *inermis* Bunge), kanzo (*Glycyrrhiza uralensis* Fisch), and shokyo (Roscoe), and may improve various IBS symptoms [11]. However, there have been few high-quality randomized controlled trials of the use of Kampo medicine for IBS.

Many previous reports of IBS treatment trials have focused on clinical symptoms. However, as noted above, IBS symptoms are diverse. Furthermore, the most important consequence of IBS is a decrease in patients' QOL. Therefore, the most suitable endpoint of an IBS trial is the QOL of IBS patients.

We previously conducted a single-arm trial and reported that KST has a positive effect on QOL in IBS patients. Thus, we devised a phase 3, multicenter, double-blinded, randomized controlled trial to evaluate the effect of KST on QOL in IBS patients. This will be the first clinical trial of KST treatment for QOL in IBS patients.

Methods

Trial design

This trial will be a multicenter, double-blind, placebo-controlled, randomized controlled trial using patients who fulfil the Rome IV criteria for IBS [1]. Patients will be recruited from the gastroenterology outpatient clinics of Yokohama City University Hospital, Fujita Health University Hospital, Kawasaki Medical University Hospital, Aichi Medical University Hospital, Hiratsuka City Hospital, and Yokosuka General Hospital. The coordinating office will be at Yokohama City University Hospital and registration, randomized allocation, and data collection will be conducted at this site.

Ethical considerations and registration

The study protocol is in compliance with the Declaration of Helsinki [12] and the Ethics Guidelines for Clinical Research published by the Ministry of Health, Labour and Welfare, Japan. We obtained approval for this study from the ethics committee of Yokohama City University Hospital on December 16, 2016. The protocol and informed consent forms were approved by the institutional ethics committee at each of the participating institutions. This trial has been registered in the University Hospital Medical Information Network Clinical Trials Registry as UMIN000026235. Written informed consent for participation in the study will be obtained from all participating patients. The trial results will be reported in conformity with the Consolidated Standards of Reporting Trials 2010 guidelines [13].

Participants

Eligibility criteria: Patients who are diagnosed with IBS according to the Rome IV criteria [1,4], were previously diagnosed as IBS according to the Rome III criteria [14], and who have received IBS treatment will be recruited for this study.

The Rome IV criteria for IBS are as follows:

Recurrent abdominal pain, on average at least 1 day per week in the last 3 months, associated with two or more of the following criteria:

1) Related to defecation. 2) Associated with a change in frequency of stool. 3) Associated with a change in form (appearance) of stool.

These criteria should be fulfilled for the last 3 months, with symptom onset at least 6 months before diagnosis [1,4].

IBS has been classified into four subtypes according to the predominant bowel pattern: IBS-C, IBS-D, IBS-M, and IBS-U. IBS subtypes will be established according to stool consistency using the Bristol Stool Form Scale [15].

The other proposed inclusion criteria for the study are as follows:

1) Aged 20 to 79 years on the date of informed consent. 2) Absence of mechanical disorders confirmed by colonoscopy within 5 years before trial entry. 3) An IBS-QOL score of less than 70 [16]. 4) Willingness to provide written informed consent.

The proposed exclusion criteria are as follows:

1) History of abdominal surgery within 6 months before trial entry. 2) Concurrent serious cardiovascular, respiratory, renal, hepatic, gastrointestinal (excluding IBS), blood, or neurological diseases. 3) History or current evidence of celiac disease or inflammatory bowel disease. 4) Current treatment with steroids or biological products. 5) Current evidence of severe psychiatric diseases that could affect the evaluation of the study drug efficacy. 6) History or current evidence of drug or alcohol abuse. 7) History of herbal medicine allergies. 8) History or current evidence of lactose intolerance. 9) Administration of new drugs for any disease within 4 weeks before entry. 10) Adjustment of medication within 4 weeks before entry. 11) Administration of medication under development. 12) Enrollment in previous clinical trials of KST or previous KST consumption. 13) Administration of other Kampo medicine. 14) Current participation or previous participation in other clinical trials within 12 weeks before entry. 15) Pregnant or possibly pregnant, or wish to become pregnant during the study period. 16) Lactating. 17) Patients judged as being inappropriate candidates for the trial by the investigators.

Intervention

Patients who meet the Rome IV criteria for IBS [4], regardless of subtypes, and/or have already been diagnosed with IBS and received IBS therapy will be recruited. All patients will provide written informed consent before participating in any study-related procedures. Patients who are satisfied with the eligibility and exclusion criteria will be monitored during a 1-week baseline period during which data on their backgrounds, blood tests, IBS-QOL, and IBS-SI will be collected to ensure that patients meet the criteria. If the participants have received medications for IBS, the medications will continue during study period without additional medicine.

All eligible patients will be randomly allocated into one of two groups: the KST group or the placebo group. Patients in the KST group will receive an oral dose of 2.5 g KST three times per day before or between meals for 8 weeks. Those in the placebo group will receive placebo medicine with the same frequency as the KST group. The follow-up period will last for 2 weeks after the treatment.

All subjects will be instructed to visit the study site 4 and 8 weeks after the initiation of the therapy. Patients will complete IBS-QOL and IBS-SI measures on site at each visit.

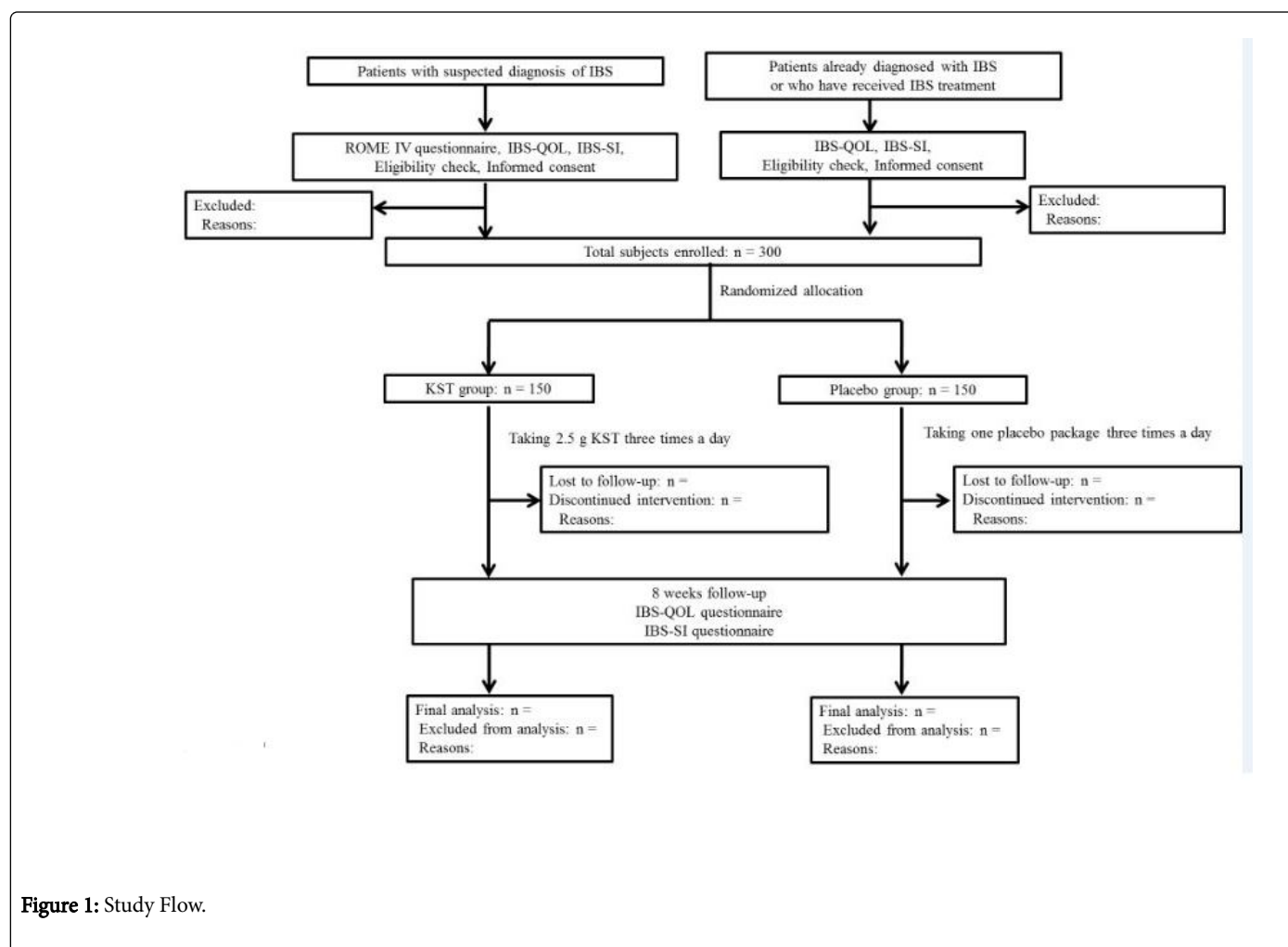


Figure 1: Study Flow.

Outcome measurements

The primary endpoint of this study will be changes in IBS-QOL overall scores after the intervention in the two groups. The IBS-QOL is a reliable and specific self-administered questionnaire developed and validated to assess QOL impairment in IBS. It comprises 34 items rated on a 5-point Likert scale (1: not at all, 2: slightly, 3: moderately, 4: quite a bit, 5: extremely or a great deal) [16].

The IBS-QOL measure contains eight subscales: dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual concerns, and relationships. The total score is converted into a score between 0 (worst) and 100 (best) points to aid interpretation, and lower scores indicated poorer QOL.

The secondary endpoints will be (for the two groups) changes in IBS-QOL overall scores after 4 weeks of intervention, changes in IBS-QOL subscale scores after 4 and 8 weeks of intervention, post-intervention changes in IBS-QOL overall scores for each IBS subtype (IBS-D, IBS-C, IBS-M, IBS-U), post-intervention changes in IBS-SI scores, adverse events, and compliance with medication. The IBS-SI was developed and validated to assess the major gastrointestinal symptoms of IBS [17]. The scoring system is simple and comprises five items rated on a 100-point scale (0: none, 100: worst), and higher score indicate greater severity of IBS.

Safety and adverse events monitoring

All participants will receive physical examinations and laboratory tests before study entry. On every visit to the outpatient clinic, body weight and blood pressure will be monitored and laboratory tests conducted (including tests of liver and renal function and an electrocardiogram).

Adverse events will be monitored by the doctor at every follow-up visit to the outpatient clinic. Adverse events will be graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 4.0. If Grade 3 or more severe adverse events appear, the follow-up doctor shall report it (them) to the coordinating office and the case will be withdrawn from the study at that point.

Randomization and key open

The investigator will convey the patient's details to the central registration center via fax. After an eligibility check, patients will be randomly assigned to receive either KST or placebo at the central registration center by a computer program, using a bloc randomization method, with stratification by institute. This will ensure that the patient assignment will be concealed from the investigator. The randomization center will allocate a numbered treatment pack to each patient, which will contain all the drugs or placebos needed for 8 weeks of administration for one patient.

Key open is not scheduled until trial finish. If Grade 3 or more severe adverse events appear, the randomization center would open the allocation to only doctor in attendance.

Drug supply

KST and the placebo will be purchased from Tsumura Co., Ltd (Tokyo, Japan). All trial drugs will be packaged identically and identified only by number. We performed blinding confirmation test about taste, smell and shape and confirmed the blindness. Subjects will be instructed to take one package of the trial drug before or between each meal every day. Compliance will be monitored by counting the remaining drug packages returned by the patients at every visit to the outpatient clinic.

Sample size estimation and interim analysis

We previously conducted a pilot KST trial (UMIN000022282) and found that patients with QOL scores of less than 80 showed an improvement in QOL scores of 13.6 ± 13.7 (QOL change; average \pm standard deviation (SD)) from 59.3 ± 20.3 (baseline QOL score) after KST administration for 8 weeks (unpublished data). Previous IBS randomized controlled trials [18-22] indicate that patients with QOL scores of less than 80 showed an improvement in QOL scores of about 10 ± 3 (QOL change; average \pm SD) after placebo administration for 8 weeks. As there is a large difference in these two sets of SDs, we aimed for a larger sample size to detect the 4-point difference in QOL changes between the KST group and placebo group. We used Student's t-test with 8.5 SD, a two-sided significance level of 5%, and a power of 80% and estimated that a sample size of 142 patients per group would be necessary.

	Study Period				
	Enrolment	Allocation	Post-Allocation		Close-Out
Time point	-4w~0	0	4w	8w	10w
Enrolment					
Eligibility Screen	X				
Informed Consent	X				
IBS-QOL Questionnaire	X				
Allocation		X			
Interventions					
7.5 G KST Per Day					
Placebo Package					
Assessments					
IBS-QOL Questionnaire	X		X	X	
IBS-SI Questionnaire	X		X	X	X
Adverse Events			X	X	X
Compliance			X	X	

Table 1: The schedule of enrolment, interventions, and assessments.

Assuming some dropout and accidental error, we propose to recruit 150 patients per group (i.e., a total of 300 patients). However, because our SD estimation is uncertain, we will conduct an interim analysis to

re-estimate the sample size using the estimated SD of the interim data (18). We will conduct the interim analysis according to the following steps: 1) When the number of participants in each group reaches 75,

we will estimate the SD of QOL score variation from all cases. 2) Then we will recalculate the sample size using the estimated SD value. 3) If the re-estimated sample size is less than 150 in total, we will assume we have enough information and the trial will be terminated. If the re-estimated sample size is more than 150 and less than 300, the trial will continue until the number of participants reaches the re-estimated sample size. If the re-estimated sample size is over 300, the trial will continue up to 300 participants, as originally planned. Final analysis will be conducted without consideration of the interim analysis and the same analysis will be performed as would be performed in the absence of an interim analysis [23]. The interim analysis will be conducted by a trial statistician independent of the principal investigator and other co-investigators, the results will be evaluated by the Independent Data Monitoring Committee, and the final judgment will be reported to the principal investigator.

Statistical analysis

Analysis population will be per protocol set. Per protocol set defined as among full analysis set, population who have serious deviation from protocol. Changes in QOL scores between the baseline and 8 weeks, the primary endpoint, will be compared for the KST group and placebo group using Student's t-test. Safety, one of the secondary endpoints, will be compared using the chi-square test. Changes in IBS-SI scores between the baseline and 8 weeks will be compared for the KST group and placebo group using Student's t-test. The other endpoints will be compared using the Mann-Whitney U test or Student's t-test. A P value of <0.05 will be regarded as statistically significant.

Trial steering committee and data monitoring committee

The Trial Steering Committee and the Independent Data Monitoring Committee will be located at the Department of Biostatistics, Yokohama City University School of Medicine and Yokohama City University Center for Novel and Exploratory Clinical Trials. The management team will monitor the trial progress and data by telephone contact and/or mail and/or web conference with each of the six sites every month. If the monitoring committee decides that on-site monitoring is needed, the monitoring member will visit the site and perform face-to-face monitoring.

Study flow and the schedule of enrolment, interventions, and assessments.

A flow chart of the study is shown in Figure 1. The schedule of the study is shown in Table 1.

Discussion

This will be the first study to assess the efficacy of KST treatment for QOL in IBS patients. The constituents of KST are thought to have beneficial effects on IBS symptoms: however, its mechanism of efficacy is still unknown. KST contains various natural ingredients, such as shakuyaku, keihi, taisho, kanzo, and kyosho, and is thought to have beneficial effects on IBS symptoms. For example, shakuyaku (*Paonia lactiflora* Pall) contains paeoniflorin, oxypaeoniflorin, and benzoypaeoniflorin. Paeoniflorin shows sedative, antispastic, and analgesic effects by suppressing gastrointestinal smooth muscles. Keihi (*Cinnamomum cassia* Blume) contains cinnamic aldehyde and methoxycinnamic aldehyde and has hypotensive, sedative, and antispastic effects [24-26]. However, little is known about the interactions between the constituents of KST. The explication of the

mechanisms of Kampo medicine has just begun and more evidence is needed of its clinical effectiveness for IBS. More high-quality clinical trials to validate the use of Kampo medicine for IBS are required.

Evidence of the effectiveness of KST for IBS would expand therapeutic options for IBS and have a substantial clinical impact. We therefore consider it important to determine whether KST can improve the QOL of IBS patients.

Declarations

Ethics approval and consent to participate

We obtained approval for this study from the ethics committee of Yokohama City University Hospital on December 16, 2016. The protocol and informed consent forms were approved by the institutional ethics committee at each of the participating institutions. Written informed consent for participation in the study will be obtained from all participating patients.

Consent for Publication

Written informed consent for publication will be obtained from all participating patients.

Availability of data and materials

The datasets used and/or analysed during the current study will be available from the corresponding author on reasonable request. All data generated or analysed during this study are included in this published article (and its supplementary information files).

Competing interests

All the authors disclose that there are no conflicts of interest relevant to this trial.

Funding

This trial is sponsored by Research for creating scientific knowledge about Kampo medicine from the Japan Agency for Medical Research and Development.

Authors' contributions

TH, AF and AN conceived the study. TH and AF conducted feasibility phase work. HO, YK, SG, LT, NO, TI, AM, NM, KH, MN, YN, NO, YF, SY, YF and KK will recruit participants and conduct follow-up at the outpatient clinic. Analysis and interpretation of data will be conducted by MT. HI and MI will carry out trial monitoring and data management. All authors have read and approved the final manuscript.

Acknowledgments

We thank Diane Williams, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

References

1. Drossman DA (2016) Functional Gastrointestinal Disorders: History, Pathophysiology, Clinical Features, and Rome IV. *Gastroenterol* 150: 1262-1279.

2. Soares RL (2014) Irritable bowel syndrome: a clinical review. *World J Gastroenterol* 20: 12144-12160.
3. Lovell RM, Ford AC (2012) Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clin Gastroenterol Hepatol* 10: 712-721.
4. Lacy BE, Mearin F, Chang L, Chey WD, Lembo AJ, et al. (2016) Bowel Disorders. *Gastroenterol* 150:1393-1407.
5. Singh P, Staller K, Barshop K, Dai E, Newman J, et al. (2015) Patients with irritable bowel syndrome-diarrhea have lower disease-specific quality of life than irritable bowel syndrome-constipation. *World J Gastroenterol* 21: 8103-8109.
6. McFarland LV (2008) State-of-the-art of irritable bowel syndrome and inflammatory bowel disease research in 2008. *World J Gastroenterol* 14: 2625-2629.
7. Dorn SD, Morris CB, Schneck SE, Hopper TM, Hu YJ, et al. (2011) Development and validation of the irritable bowel syndrome satisfaction with care scale. *Clin Gastroenterol Hepatol* 9:1065-71.
8. Yu F, Takahashi T, Moriya J, Kawaura K, Yamakawa J, et al. (2006) Traditional Chinese medicine and Kampo: a review from the distant past for the future. *J Int Med Res* 34: 231-239.
9. Watanabe S, Imanishi J, Satoh M, Ozasa K (2001) Unique place of Kampo (Japanese traditional medicine) in complementary and alternative medicine: a survey of doctors belonging to the regional medical association in Japan. *Tohoku J Exp Med* 194:55-63.
10. Shumer G, Warber S, Motohara S, Yajima A, Plegue M, et al. (2014) Complementary and alternative medicine use by visitors to rural Japanese family medicine clinics: results from the international complementary and alternative medicine survey. *BMC Complement Altern Med* 14: 360.
11. Miura S, Sugano K, Kinoshita Y, Fock KM, Goh KL, et al. (2011) Diagnosis and treatment of functional gastrointestinal disorders in the Asia-Pacific region: A survey of current practices. *J Gastroenterol Hepatol* 26: 2-11.
12. World Medical Association (2013) Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 310: 2191-2194.
13. Schulz KF, Altman DG, Moher D (2010) CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340: c332.
14. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, et al. (2006) Functional bowel disorders. *Gastroenterology* 130: 1480-1491.
15. Lewis SJ, Heaton KW (1997) Stool Form Scale as a Useful Guide to Intestinal Transit Time. *Scand J Gastroenterol* 32: 920-924.
16. Patrick DL, Drossman DA, Frederick IO, DiCesare J, Puder KL (1998) Quality of life in persons with irritable bowel syndrome: development and validation of a new measure. *Dig Dis Sci* 43: 400-411.
17. Francis CY, Morris J, Whorwell PJ (1997) The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Alimentary pharmacology & therapeutics* 11: 395-402.
18. Lorenzo-Zuniga V, Llop E, Suarez C, Alvarez B, Abreu L, et al. (2014) I. 31, a new combination of probiotics, improves irritable bowel syndrome-related quality of life. *World J Gastroenterol* 20: 8709-8716.
19. Fukudo S, Kinoshita Y, Okumura T, Ida M, Akiho H, et al. (2016) Ramosetron Reduces Symptoms of Irritable Bowel Syndrome With Diarrhea and Improves Quality of Life in Women. *Gastroenterol* 150:358-366.
20. Fukudo S, Ida M, Akiho H, Nakashima Y, Matsueda K (2014) Effect of ramosetron on stool consistency in male patients with irritable bowel syndrome with diarrhea. *Clin Gastroenterol Hepatol* 12: 953-959.
21. Shavakhi A, Minakari M, Farzammia S, Peykar MS, Taghipour G, et al. (2014) The effects of multi-strain probiotic compound on symptoms and quality-of-life in patients with irritable bowel syndrome: A randomized placebo-controlled trial. *Adv Biomed Res* 3: 140.
22. Abbasnezhad A, Amani R, Hajiani E, Alavinejad P, Cheraghian B, et al. (2016) Effect of vitamin D on gastrointestinal symptoms and health-related quality of life in irritable bowel syndrome patients: a randomized double-blind clinical trial. *Neurogastroenterol Motil* 28: 1533-1544.
23. Proschan MA (2005) Two-stage sample size re-estimation based on a nuisance parameter: a review. *J Biopharm Stat* 15: 559-574.
24. Kito Y, Teramoto N (2012) Effects of Hange-shashin-to (TJ-14) and Keishi-ka-shakuyaku-to (TJ-60) on contractile activity of circular smooth muscle of the rat distal colon. *Am J Physiol Gastrointest Liver Physiol* 303: 1059-1066.
25. Nobe K, Momose K, Sakai Y (2002) Effects of Kampo medicine, keishi-ka shakuyaku-to (TJ-60) on alteration of diacylglycerol metabolism in gastrointestinal smooth muscle of diabetic rats. *Acta pharmacolog Sinica* 23:1173-1180.
26. Saitoh K, Kase Y, Ishige A, Komatsu Y, Sasaki H, et al. (1999) Effects of Keishi-ka-shakuyaku-to (Gui-Zhi-Jia-Shao-Yao-Tang) on diarrhea and small intestinal movement. *Bio Pharma Bulletin* 22:87-89.