PROTOCOL

Supplementation with a multi-strain probiotic formulation (Bio-Kult®) in the management of diarrhoea-predominant irritable bowel syndrome - a randomized, double-blind, placebo-controlled clinical trial

PREPARED BY

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ABSTRACT

Background: Irritable Bowel Syndrome (IBS) is a common functional gastrointestinal disorder. Common clinical manifestation include abdominal pain, bloating, altered bowel habit which bears a great impact on the quality of life of an individual. Previously limited number of studies carried out in our country to assess the effect of probiotics on IBS patients which yielded mixed results. Further study preferably with larger sample and longer duration was recommended. Aims and objectives: This study aims to see the effect of probiotics on IBS.

Material and method: This is a controlled randomized clinical trial. The patient will be selected on the basis of Rome III criteria. IBS patients with a sample size of 400 will be taken. Purposive non probability method of sampling will be employed. In this method the patients who will be readily available and in close proximity will be selected according to the selection criteria. Patient will be collected from gastroenterology outpatient department. After full explanation of the study procedure, informed written consent will be taken. Participants will be randomized to receive either probiotics (Biocult®, Sandooz Bd. Ltd.) with standard treatment or only standard treatment for 8 weeks. All participants will be interviewed with baseline symptom score. Similar interview for symptom score will take place at 1 month interval for 3 times (including 1 month post treatment). All data will be processed by the SPSS program values will be expressed as frequencies or mean±SD unless otherwise mentioned. Between groups analysis will be done by Chi-square test, unpaired or paired students t-test as applicable; while predictive values will be seen by regression analysis. Only P values <0.05 will be considered as significant.

Result: This study will see the effect of two months use of probiotics on IBS and thereby highlight over the longer treatment of IBS.

INTRODUCTION & BACKGROUND

Irritable Bowel Syndrome (IBS) is the most common functional disorder in clinical practice (Thompson WG et al.1992). IBS is characterized by abdominal pain, bloating and change in stool frequency and consistency in the absence of an organic cause. Although the prevalence has not been firmly established, it has been estimated that IBS affected 14-24% of women and 5-15% of men in western countries. The worldwide prevalence is approximately 10- 20% of the adult (Drossman et al.1993). IBS was shown to be more common in females in a rural community in Bangladesh. (Masud MA et al, 2001) Although the one year prevalence is about 19%, only a third present to their GPs (Jones R, Lydeard S, 1992). If the strict Rome III criteria for IBS are used, it affects around 5-11% of individuals, with similar prevalence in developed or developing countries (Guidelines--Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders, 2006).

IBS may present at any age with peak prevalence in 30s and 40s - female predominance is most obvious in the 3rd decade and declines afterwards. (Spiller R et al, 2006). Symptoms may arise from the whole gut rather than just the colon. There is no structural lesion, but there appears to be a significant subgroup of patients in whom IBS is precipitated by an episode of bacterial gastroenteritis (Neal KR et al, 2002).

The pathogenesis of IBS is multi factorial, including roles for genetics, abnormal pain processing, behavioral pathways and the gastrointestinal microbiota (Chang JY et al. 2011). No specific diagnostic procedures identify IBS, because the underlying pathophysiology remains unknown. Thus, the diagnosis is dependent on symptoms and exclusion of major organic causes (Thompson WG et al.1989). A large case–

control study (Porter CK et al.2011) demonstrated that infectious gastroenteritis resulted in almost a fourfold increase in the odds of developing IBS within the subsequent 2 years. An imbalanced microbiota may contribute to GI symptoms through altered colonic fermentation resulting in increased formation of gas, an abnormal pattern of short chain fatty acid production and motility or sensitivity disturbances of the intestinal tract. The symptoms are not specific for IBS. The diagnosis of IBS rarely alters over time, but one always needs to be prepared to reconsider the diagnosis if the clinical picture changes. IBS has a significant negative impact on quality of life and social functioning in many patients, but it is not associated with the development of serious disease or with excess mortality. Although IBS is still considered as a functional disorder, there is increasing evidence that organic disease of the gastrointestinal tract (IBD), bacterial overgrowth, altered serotonin level and central dysregulation can be identified in subsets of patients.

Probiotics are supplements that are made up of live strains of beneficial bacteria. The Food and Agriculture Organization of the WHO provides the most widely accepted definition of probiotics as ‘a live organism that, when ingested in adequate amounts, exerts a health benefit to the host’ (FAO/WHO, 2001). There is growing body of opinion that the micro flora make up of the human body affects the overall health and risks for subsequent diseases. An imbalance of gut bacteria are thought to contribute to IBS symptoms. (Pimentel M et al. 2006).Restoration on the micro flora to normal conditions might reduce symptoms in those who suffer from IBS. Probiotics promote health by their ability to improve the micro flora in the gut, increasing numbers of beneficial species and reducing numbers of pathogenic bacteria.. Probiotics increase in trophic responses and regulate intestinal motility. Probiotics also modulate luminal

immunity by changing cytokine and cellular milieu from a pro inflammatory to anti inflammatory state. Probiotics regulate fermentation of non-degradable dietary fibre, intraluminal mucoproteins, favour lactose digestion and modulate intraluminal gas production (Douglas LC, Scanders ME, 2008). Evidence has accumulated to suggest the efficacy of certain probiotics which may be capable of bringing about a significant reduction in pain; abdominal distension and flatulence, while increasing health-related quality of life in IBS (M. Bixquet Jimenez, 2009). Several studies with L. acidophilus, S. thermophilus, L. plantarum have shown improvement of bowel symptoms in IBS patients (Sen S et al, 2002).

RATIONALE OF THE STUDY

Many drugs have been advocated in the treatment of IBS, for instance, spasmolytics, bulking agents, psychotropic agents, and5-HT receptor antagonists. However, in many cases all these options remain disappointing for the relief of symptoms. The therapeutic efficacy in IBS is probably impacted by the heterogeneous pathogenesis of the disease which includes altered intestinal motility, visceral hypersensitivity, abnormal brain gut interaction, food intolerance, altered intestinal permeability and post infectious changes. Recently, the adverse role of alteration of gut micro flora at the onset of symptoms has been emphasized. Therefore, rationale exists for the therapeutic use of probiotics in IBS. In 2004-2005 a randomized, double blind placebo controlled trial (Kabir MA et al, 2011) on the effect of S. boulardii in diarrhea predominant IBS patients was conducted at BSMMU but the result was not satisfactory. Another study (Rahman MZ et al, 2013) at BSMMU during 2010-2011 with multistrain probiotics yielded a beneficial outcome which was significant both clinically and statistically. On both occasions, further study was suggested preferably with a larger sample size. These recommendations for further study prompted this study to be carried out in order to assess the effect of multistrain probiotics in relation to change of symptom scores in IBS patients and also to see changes of quality of life before and after the treatment. As per EMEA guideline, previous study (Spiegel B et al, 2009) revealed that abdominal pain is the most prevalent and distressing symptom in IBS patients and changes in the severity of pain were reflected as a more reliable outcome of successful treatment. IBS shouldn't be diagnosed in the absence of abdominal pain or discomfort. The pain or discomfort is typically relieved by

defecation or its onset is associated with an increased or decreased frequency of stool or looser or harder stool. The pain often is poorly localized, waxes and wanes, may be aggravated by eating & can occur in any part of abdomen; although it is more typically located in the lower abdomen. It can be referred to different areas in the abdomen or to the chest or back. Exacerbation of pain by stressful life events or difficult life situation is common. As abdominal pain is the hallmark feature of IBS and is recommended for the time being to assess the main symptomatology in at least partially validated scale. So, improvement of abdominal pain or discomfort along with abnormalities in defecation is considered as primary outcome. For other subtypes of IBS, and for "global" development programs intending to treat two or more subtypes, the use of the global assessment is, however, still recommended. Both endpoints should be evaluated as responder rates. The numerical evaluation of changes in scales is regarded to be a secondary endpoint. As per EMEA Guidance on The Evaluation of Medicinal Products for the Treatment of IBS, a responder has to demonstrate abdominal pain score which has improved at least 30% compared to baseline. Recent studies also show that there is high placebo response rate in functional bowel disorders which has a negative correlation with duration of study. So to overcome this confusion of high placebo response, this study is designed, unlike previous studies in our country, with a longer study period along with a larger sample size.

RESEARCH QUESTION

Is multistrain probiotics with standard treatment more effective at reducing IBS symptoms than standard treatment of IBS?

OBJECTIVES OF THE STUDY

Primary Objectives:

1. To assess the effect of multistrain probiotics on abdominal pain using a validated symptom severity score in IBS patients.
2. To assess the efficacy of a multi-strain probiotic supplement as a treatment option for IBS in a tertiary referral centre

Secondary Objectives:

1. To assess the effect of probiotics on global symptom severity scores which includes other symptoms of IBS (stool consistency, frequency, bloating) using a validated symptom severity score.
2. To assess changes in the use of rescue medication (i.e. laxatives or anti diarrhea medication such as Loperamide).
3. To assess any adverse events reported with probiotic use.

MATERIALS AND METHODS

a. Study Population:

Diagnosed cases of IBS using Rome III criteria.

Ethnicity - Bangladeshi

Age - 18-55 years

Sex - Male and female

b. Study design: Randomized controlled clinical trial.

c. Main outcome variables to be studied:

Changes in abdominal pain for primary objective and overall symptom severity score as a secondary objective.

d. Checklist of Variables:

Height Weight

BMI DM

Psychological disorder(depression, anxiety)

e. Screening method:

Subjects will be screened by FBC, ESR, CRP, Coeliac serology following Rome III criteria for diagnosis of IBS.

f. Eligibility:

g. Inclusion Criteria:

1. Diagnosed case of IBS using Rome III criteria
2. Absence of red flag sign: anemia, fever, wt loss, per rectal bleeding, nocturnal frequency, family history of IBD, cancer
3. Age 18-55 years
4. No probiotics used in prior 3 months.
5. Agreed not to start any other drug unless clinically indicated.
6. No antibiotics in previous 2 months of enrolment.

h. Exclusion Criteria:

1. Age<18 or >55 years
2. Previous treatment with probiotics within last 3 months
3. Pregnant or lactating lady
4. Concurrent severe illness (Uncontrolled DM, Renal Dysfunction, Liver disease, hyper and hypothyroidism)
5. Chronic organic bowel disorders e.g. inflammatory bowel diseases, TB, Diverticular disease etc
6. Any previous gastrointestinal surgery

i. Sample Size:

For our trial, we will take equal sample size for both the groups ($n_1 = n_2$). The sample size is to be determined using the following formula -

Sample size in each group, $n_1 = 2\ \sigma^2(Z_\beta + Z\alpha)^2 / (\mu_1 - \mu_2)^2$

Here,

Z_β= 0.84 at 80% power

$Z\alpha$ = 1.96 at 95% confidence interval

$\mu_1 - \mu_2$= Minimum clinically important difference = 30 %(a minimal clinically significant reduction of 30% is desirable in probiotics group compared to placebo)

σ = Standard deviation = 87.77 (G. Sisson, et al. 2013)

Since there is no such study or trial carried out in Bangladesh, we could not find any reference value of Standard deviation of IBS-QOL or IBS-SSS scale among patients of Bangladesh. Hence we used a recent study (G. Sisson, 2013) to estimate the standard deviation.

The standard deviation found in the G. Sisson, et al. 2013 trial was 13.21 for IBS-QOL scale and 87.77 for IBS-SSL. Our study objectives include both the measures; hence we have considered the higher value of standard deviation so that the sample size is sufficient to cover both objectives.

Sample size in each group, $n_1 = 2 \times (87.77)^2 \times (0.84 + 1.96)^2 / (30)^2$

$= 135$

Total sample size, n = 270

Adjustment for non-compliance/cross-over

We also need to consider dropouts and poor adherence in calculating the sample size. It is estimated that around 5%-8% patients in both groups will drop out in different stages of the trial. The adjusted sample size is calculated based on the following formula –

Adjusted sample size, $n_{adj} = n/(1-c_1-c_2)^2$

Here, n = 270

c_1 = Dropout rate in control group = 8%

c_2= Dropout rate in control group = 8%

Adjusted sample size, $n_{adj} = 270 / (1-.08-.08)^2$

$= 384 \approx 400$

The adjusted sample size in each group = 200

j. Operatinal definition:

Rome III criteria for diagnosing IBS

Recurrent abdominal pain or discomfort (an uncomfortable sensation not described as pain) at least three days a month in the past three months, associated with two or more of the following:

- Improvement with defecation.
- Onset associated with a change in frequency of stool.
- Onset associated with a change in form (appearance) of stool.

Criteria should be fulfilled for the past three months with symptom onset at least six months before diagnosis.

IBS patients will be diagnosed by translated and validated enhanced Asian ROME III questionnaires in Bengali language for diagnosis of IBS. (Mohammed et al, 2014).

Outcome variables:

According to the EMEA Guidance on the Evaluation of Medicinal Products for the Treatment of IBS, a responder is defined as a patient one whose abdominal pain score has improved at least 30 % compared to baseline.

Confounding variables:

These are the factors that may influence the course of the study and modulate the findings on interpretations.

Following are the confounding variables in this study and these will be addressed accordingly:

1. Psychological status- IBS is a functional gastrointestinal disorder and by definition psychological attributes i.e. anxiety and depression may influence the appearance and/or severity of pain and bowel motion. Such patients in this study

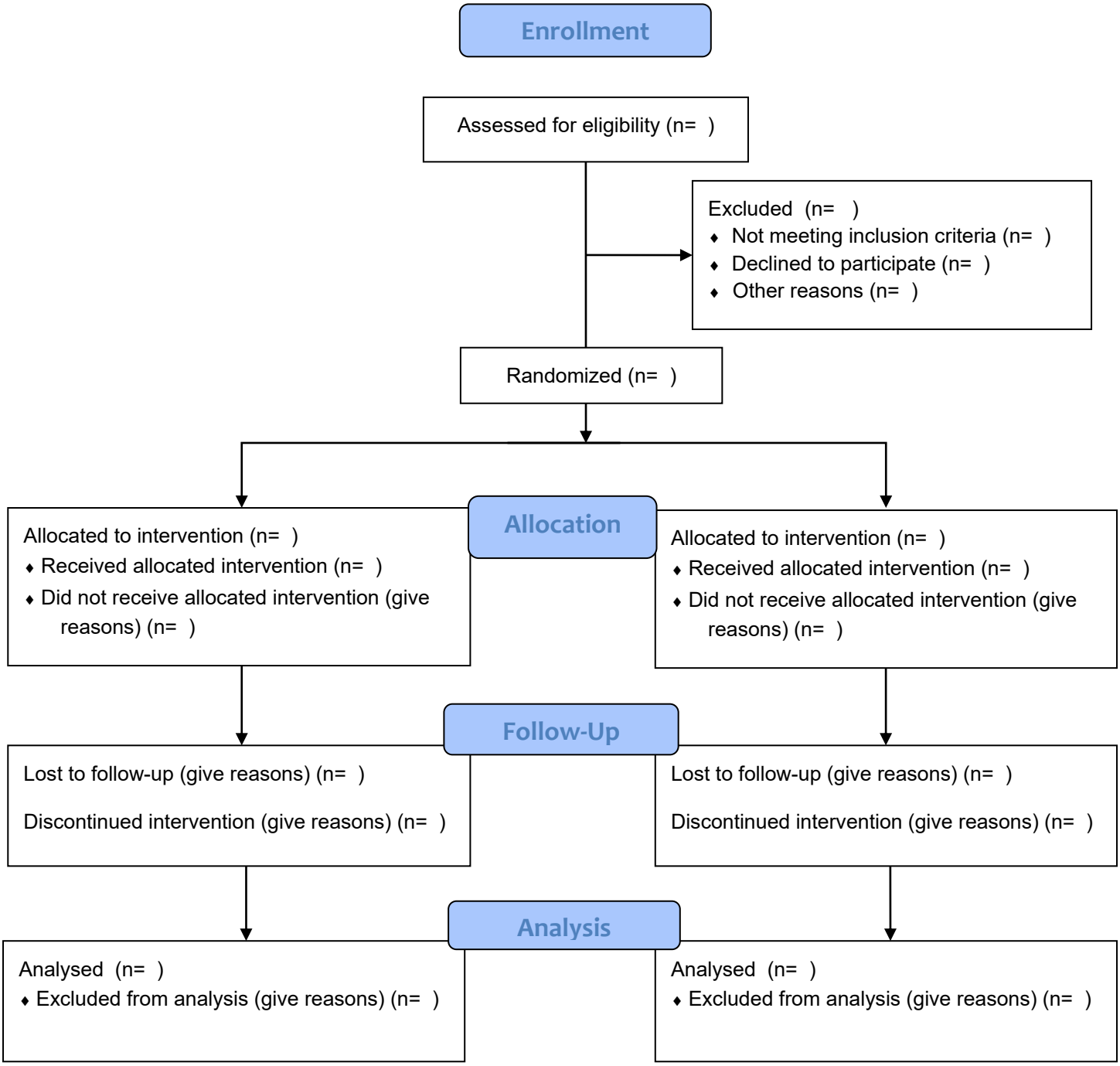
will be managed by anxiolytics and the antidepressants in appropriate cases so that their psychological status does not influence the outcome of the study.

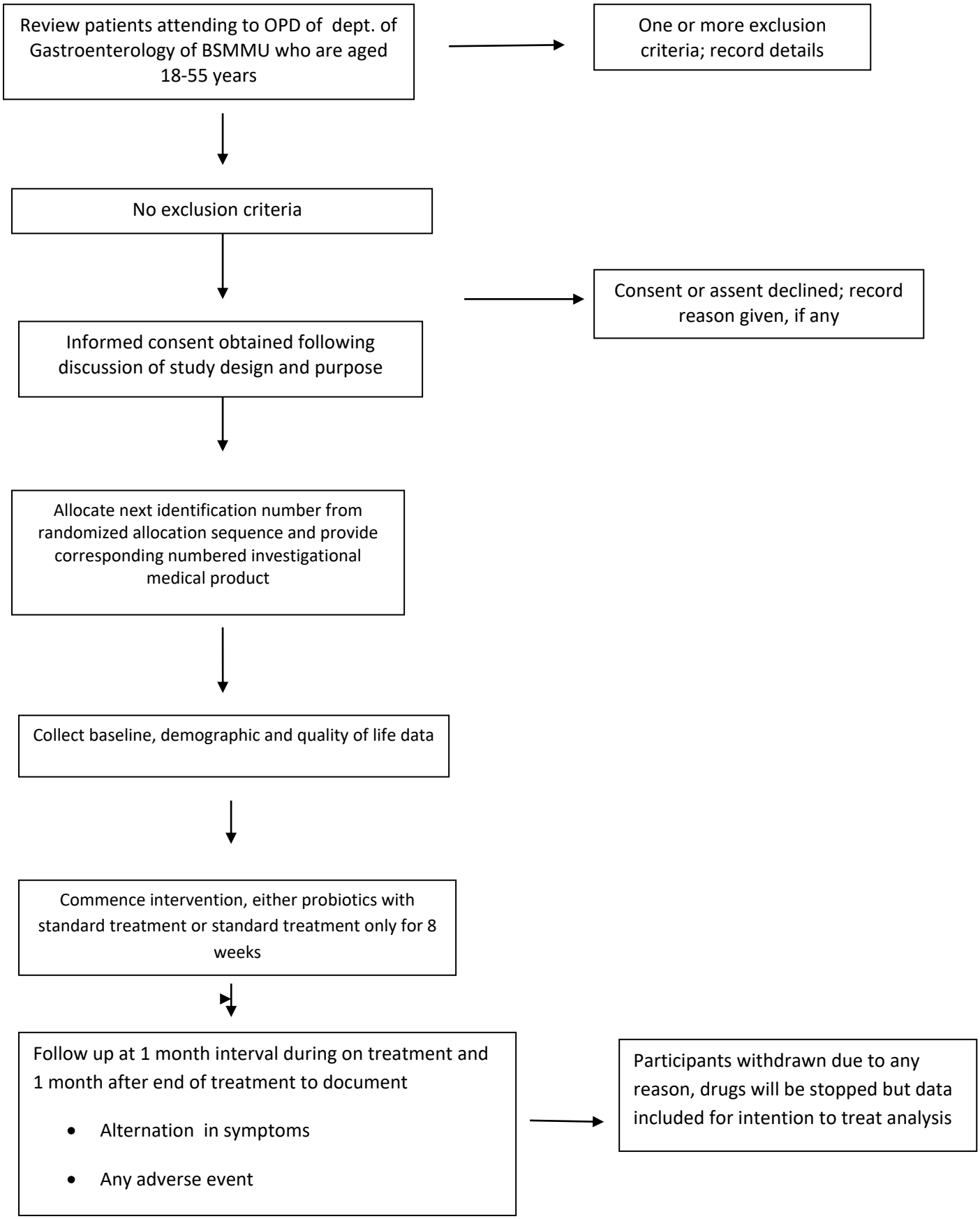
2. Dietary factors: Some dietary components may aggravate abdominal pain and frequency of bowel motion in IBS. Patient of both groups will be provided with similar dietary advices. So that dietary component can not influence the outcome of the study.
3. Drugs: Spasmolytics, antidiarrhoeal and bulk forming agents will be prescribed in both the groups similarly according to the needs of the patients. So that they do not influence the outcome of the study.

Follow up schedule:

Participants will be followed up before intervention for baseline information and 1 month interval during treatment including 1 month post treatment**.**

k. Flow chart showing the sequence of tasks:




Review patients attending to OPD of dept. of Gastroenterology of BSMMU who are aged 18-55 years
One or more exclusion criteria; record details
No exclusion criteria
Consent or assent declined; record reason given, if any
Informed consent obtained following discussion of study design and purpose
Allocate next identification number from randomized allocation sequence and provide corresponding numbered investigational medical product
Collect baseline, demographic and quality of life data
Commence intervention, either probiotics with standard treatment or standard treatment only for 8 weeks
Follow up at 1 month interval during on treatment and 1 month after end of treatment to document
Alternation in symptoms
Any adverse event
Participants withdrawn due to any reason, drugs will be stopped but data included for intention to treat analysis

l. Procedures of preparing and organizing materials:

In people who meet the IBS diagnostic criteria, the following tests should be undertaken to exclude other diagnoses:

- Full blood count (FBC)
- Erythrocyte sedimentation rate (ESR) or C - reactive protein (CRP)
- Antibody testing for coeliac disease
  (Endomysial antibodies [EMA] or tissue transglutaminase [TTG]).

Participants who fulfill the inclusion criteria in the absence of exclusion criteria will be included in the study. IBS patients will be diagnosed by translated and validated enhanced asian ROME III questionnaires in Bengali language for diagnosis of IBS. (Mohammed et al, 2014) Informed written consent will be obtained from participants. Data will be collected in questionnaire.

All the participants are advised to remain on their usual normal diet during the study period. Approximately total of 400 participants will be recruited in this study. They will be divided into two groups by randomization software, consisting of 200 in each group. One group will receive two capsules of probiotics (Bio-Cult, Sandors BD Ltd.) twice daily before or during a meal with standard treatment, while the other group will receive only standard treatment for 8 weeks.

Bio-Cult is a 14 strain probiotic formulation in a capsule form. Two capsules twice a day is equivalent to 8 billion CFUs (2 billion per capsule). The strains are-
[Bacillus subtilis PXN 21, Bifidobacterium spp. (B. bifidum PXN 23, B. breve PXN 25, B. infantis PXN 27,B. longum PXN 30), Lactobacillus spp. (L. acidophilus PXN 35, L. delbrueckii spp. Bulgaricus PXN39, L. casei PXN 37, L. plantarum PXN 47, L. rhamnosus PXN 54 , L.helveticus PXN 45,L. salivarius PXN 57) Lactococcus Lactis PXN 63, Streptococcus thermophilus PXN 66].
Changes of symptoms will be assessed by using a previously used validated IBS instrument. (Richard Lea et al.2001). Symptom severity scoring system of IBS is shown in diagram-1 (C.Y. Francis et al. 1997). Symptom severity scores specially for abdominal pain, global symptom severity will be assessed as baseline and at 1 month

interval during treatment along with 1 month post treatment. This scoring will be done on the basis of monthly interviews.

The collective scores to these individual domains give rise to the total score. The IBS-SSS total score ranges from 0 to 500; a higher score indicating worse condition. Scores below 175 represent mild IBS, 175–300 represent moderate severity, and scores above 300 represent severe IBS.

Patients will be asked to keep a diary with details of use of concomitant medication provided used consistently over last 2 months with a stable dose i.e. Loperamide or laxative including amount, frequency and duration.

All data will be recorded in a printed data sheet.

m. Randomization:

Randomization procedure will be done by randomizer software (www.randomizer.org)

n. Equipments to be used:

For data collection: Data collection sheet.

o. Assurance of compliance in trial:

The patient will be motivated for compliance. They will be assessed for willingness before selection. Adequate information will be provided regarding dose and duration of drugs. Patient will be followed up monthly for 3 months for improvement and also for compliance. Compliance will be assessed by counting the foils/boxes of use medication. Compliance will also assessed by asking about other medications and dietary patterns of the patient.

p. Procedure of data analysis and interpretation:

Data will be analyzed using computer based SPSS program (version 13.0). All data will be expressed as frequencies and mean (±SD or ± SE). Comparison of symptom score between subgroups will be done by Student's unpaired t-test. Pearson's correlation test will be used to see correlation among different variables. P values less than or equal to 0.05 will be considered as significant.

q. Intention to treat analysis:

After randomization, some patient may become non complaint to the allocated treatment and will be lost to follow-up. Randomization is a key process in a randomized controlled trial. So exclusion of this non complaint and drop out patients from the analysis of the result may create bias, reduce the sample size and thereby reduce the power of statistical tests and increase the probability of type II error.

Treatment group and control group will be analyzed with respect of their random allocation regardless of what will happen subsequently (whether they will actually receive the treatment or not).

r. Time table:

	(Activities with time schedule)
Topic selection:	03 month (Sept-Nov, 2014)
Literature search:	All through the study period
Protocol writing and approval:	03 months (Dec-Feb, 2014)
Data collection:	12 months (Apr, 2015 –Mar, 2016)
Data analysis:	01 month (April, 2016)
Thesis writing:	02 months (March-April, 2016)
Revision, binding and submission:	02 months (May-June, 2016)

s. Good clinical practice:

According to the principles of GCP developed by International Conference on Harmonisation (ICH) following principles will be ensured in this study -

- Data and reported results of clinical investigations are credible and accurate, and
- Rights, safety and confidentiality of participants in clinical research are respected and protected (Guidance for Implementation. WHO, 2002).

Ethical Consideration:

a) Before starting this study, the research protocol was submitted and approved by the Ethical Review Committee of BSMMU, Dhaka.

b) All participants will be informed about the objectives, methodology and purpose of the study in an easy understandable way.

c) All information regarding benefits and hazards will be delivered to the all participants & those who agree to participate will be included in the study.

d) Verbal and written consents will be taken from all participants without any influences prior to sample collection.

e) If the participants have any question or any other problems, they will be requested to contact.

f) Data obtained from the study will be used only for the research purpose and the confidentiality of all study information will be maintained strictly.

g) Participants can withdraw themselves from the study any time even after giving consent.

References:

Chang JY, Talley NJ. An update on irritable bowel syndrome: from diagnosis to emerging therapies. *Curr Opin Gastroenterol* 2011; 27:72–78.

Douglas LC, Scanders ME.Probiotics and Prebiotics in dietics practice. *J AM Diet Assoc.* 2008; 108: 510-21.

Drossman DA, Liz Andruzi E.US householder survey of functional GI disorder, prevalence, socio demography and health impact. *Dig Dis Sci.* 1993; 38:1569-80.

FAO/WHO. FAO/WHO 'Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria'. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria (Octobe2001).

Guidelines on the Irritable Bowel Syndrome: Mechanisms and Practical Management. *British Society of Gastroenterology*, 2007.

http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf [Accessed 23 November 2012].

Jones R, Lydeard S. Irritable bowel syndrome in the general population. *BMJ*, 1992 Jan 11; 304(6819):87-90.

Kabir MA, Ishaque SM, Ali MS, Mahmuduzzaman M, Hasan M. Role of Saccharomyces Boulardii in Diarrhea Predominant Irritable Bowel Syndrome. *Mymensingh Medical Journal.* 2011;Vol 20, No 3:397-401.

M.Bixquet Jimenez.Treatment of Irritable Bowel Syndrome with probiotics. An etiopathogenetic approach at last? *Rev Esp Dig.* 2009; 101:553-64.

Masud MA, Hasan M, Azad Khan AK. Irritable Bowel Syndrome in a rural community in Bangladesh. Prevalence, symptom pattern and health seeking behavior. *AM J Gastroenterol.* 2001; 96:1547-52.

Mohammed Rahman, Uday Ghoshal, Faruque Ahmed, Md Kibria, Mahmud Hasan, Ann Gwee et al. Translation and Validation of Enhanced Asian Rome 3 Questionnaires (EAR3-Q) in Bengali Language for Diagnosis of Functional Gastrointestinal Disorders. *AM J Gastroenterol*. October-2014; Vol-109.

Neal Kr, Barker L, Spiller RC. Prognosis in post-infective irritable bowel syndrome: a six year follows up study. Gut, 2002 Sep; 51(3):410-3.

Porter CK, Gormley R, Tribble DR, et al. The incidence and gastrointestinal infectious risk of functional gastrointestinal disorders in a healthy US adult population. *Am J Gastroenterol.* 2011; 106:130–138.

Rahman MZ, Chowdhury MS, Rahman MA, Parveen S, Barua R, Ishaque SM, Ahmed DS, Raihan ASMA. Efficacy of Probiotics in Irritable Bowel Syndrome-a randomized, double blind placebo controlled study. *BSMMU J.* Vol. 6, Issue 1, Jan 2013:21-28.

Sen S, Mullan MM, Parker TJ, Woolner JT, Tarry SA, Hunter JO. Effect of Lactobacillus plantarum 299v on colonic fermentation and symptoms of irritable bowel syndrome. *Dig Dis Sci*. 2002;47:2615-2620.

Spiller R, Aziz Q, Creed F et al.Guidelines for the management of Irritable Bowel Syndrome. Gut, 2007

Thompson WG, Dotevall G, Drossman DA, Binder V, Kreiner S et al. Irritable Bowel Syndrome: Guideline for the diagnosis. *Gastroenterol.* 1989; 2: 92-95.

Thompson WG, Gred F, Drossman da, Heaton KW, Mazzacea G. Functional bowel disorder and functional abdominal pain. *Gastroenterol.* 1992; 5:75-91.

Thompson WG, Heaton KW, Smyth GT, Smyth C. Irritable bowel syndrome in general practice: prevalence, characteristics, and referral. Gut. 2000; 46:78-82

APPENDIX - I

DATA COLLECTION SHEET

1. Baseline demographic profile of the patients: Date:

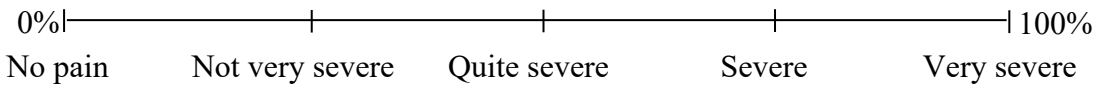
a) Group :
b) Patients ID no. :
c) Name :
d) Age :
e) Sex :
f) Religion :
g) Occupation :
h) Address :
i) Contact no. :

Symptom severity score questionnaire:

1. a) Do you currently suffer from abdominal pain?

Before treatment	At 1st month	At 2nd month	At 3rd month
□Yes/□No	□Yes/□No	□Yes/□No	□Yes/□No

b) If yes, how severe is your abdominal pain?



Before treatment	At 1st month	At 2nd month	At 3rd month

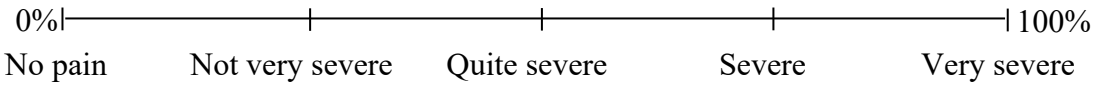
c) Please enter the number of days that you get the pain in every 10 days.

Before treatment	At 1st month	At 2nd month	At 3rd month

2. a) Do you currently suffer from abdominal distension (bloating, swollen or tight)?

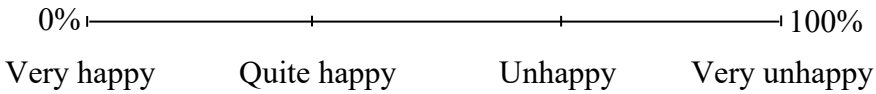
Before treatment	At 1st month	At 2nd month	At 3rd month
□Yes/□No	□Yes/□No	□Yes/□No	□Yes/□No

b) If yes, how severe is your abdominal distension/tightness?



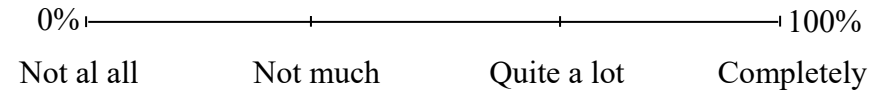
Before treatment	At 1st month	At 2nd month	At 3rd month
□Yes/□No	□Yes/□No	□Yes/□No	□Yes/□No

3. How satisfied are you with your bowel habit?



Before treatment	At 1st month	At 2nd month	At 3rd month

4. Please indicate with a cross on the line below how much your irritable bowel syndrome is affecting or interfering with your life in general.



Before treatment	At 1st month	At 2nd month	At 3rd month

5. a) What is the number of times you open your bowels per day/week/month?

Before treatment	At 1st month	At 2nd month	At 3rd month
/day	/day	/day	/day

b) What is the list number of times you open your bowels per day/week/month?

Before treatment	At 1st month	At 2nd month	At 3rd month

6. In the following questions you may circle more than one answer:

   Are your motions ever:

Before treatment	At 1st month
a) Normal : b) Hard: c) Very thin (like string): d) In small pieces (like rabbit pellets): e) Musky (like porridge): f) Watery:	a) Normal : b) Hard: c) Very thin (like string): d) In small pieces (like rabbit pellets): e) Musky (like porridge): f) Watery:
At 2nd month	**At 3rd month**
a) Normal: b) Hard: c) Very thin (like string): d) In small pieces (like rabbit pellets): e) Musky (like porridge): f) Watery:	a) Normal : b) Hard: c) Very thin (like string): d) In small pieces (like rabbit pellets): e) Musky (like porridge): f) Watery:

7. In the following questions you may circle more than one answer:

   Do you ever:

	Before treatment	At 1st month	At 2nd month	At 3rd month
a) Pass mucus (or slime or jelly) with your motions.	□Yes/□No	□Yes/□No	□Yes/□No	□Yes/□No
b) Pass blood with your motion	□Yes/□No	□Yes/□No	□Yes/□No	□Yes/□No
c) Have to hurry/rush to the toilet to open your bowels	□Yes/□No	□Yes/□No	□Yes/□No	□Yes/□No
d) Strain to open your bowels	□Yes/□No	□Yes/□No	□Yes/□No	□Yes/□No
e) Feel you haven't emptied your bowel completely after your have passed a motion	□Yes/□No	□Yes/□No	□Yes/□No	□Yes/□No

8. Do you ever:

   a) Notice your stools are more frequent or loose when you get pain..

Before treatment	At 1st month	At 2nd month	At 3rd month
□Yes/□No	□Yes/□No	□Yes/□No	□Yes/□No

b) Notice whether the pain is frequently eased by opening your bowels.

Before treatment	**At 1st month**	**At 2nd month**	**At 3rd month**
□Yes/□No	□Yes/□No	□Yes/□No	□Yes/□No

9. In the last year on approximately how many weeks were you:

   a) Absent from work due to IBS ……

   b) At work suffering from IBS ……