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Title: The Impact of faecal transplantation in the severity of Irritable Bowel Syndrome symptoms - A systematic review and meta-analysis of Randomised Control Trials.

Τίτλος: Η επίδραση της μεταμόσχευσης κοπράνων στην σοβαρότητα των συμπτωμάτων του Συνδρόμου Ευερέθιστου Εντέρου. Συστηματική Ανασκόπηση και Μετα-ανάλυση Τυχαιοποιημένων Ελεγχόμενων Μελετών.

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Title: The Impact of faecal transplantation in the severity of Irritable Bowel Syndrome symptoms - A systematic review and meta-analysis of Randomised Control Trials.

Abstract:

Background: Irritable Bowel Syndrome (IBS) is a common gut- brain axis disorder with no effective treatment so far. Dysbiosis of gut microbiota, is involved in its pathogenesis and Faecal Transplant Microbiota (FMT) has been implemented to directly modify the gut microbiota. Randomised Control Trials (RCTs) have had controversial outcomes so far.

Methods: A systematic literature review was performed in PubMed, Embase, Medline, Cochrane, and ClinicalTrials.gov databases and RTCs in adult patients with IBS who received FMT and followed up for at least 12 weeks, were included. The primary outcome was the effect of the FMT in severity of symptoms in IBS.

Results: Data at week 12 post-intervention were analysed from 7 RCTs. High heterogeneity in between studies was found (I^2 =87%). Random Effect (RE) model was used and OR was calculated as 1.9, 95% CI (0.56,6.5). In subgroup analysis, patients in the Endoscopy/NJ &fresh/frozen-thawed subgroup had I^2: 71% and the RE model analysis showed an OR: 3.97, 95% CI (1.35, 11.72).

Conclusion: No statistically significant improvement in symptoms severity with FMT was identified. In subgroup analysis, patients who received fresh or frozen-thawed samples via

endoscopy or NJ showed a statistically significant improvement, though. Therefore, more evidence from RCTs is needed for safe recommendations to be feasible.

Keywords: faecal transplant, irritable bowel syndrome (IBS), Irritable Bowel Syndrome Severity Scoring System (IBS-SSS), Faecal microbiota transplantation (FMT)

Τίτλος: Η επίδραση της μεταμόσχευσης κοπράνων στην σοβαρότητα των συμπτωμάτων του Συνδρόμου Ευερέθιστου Εντέρου. Συστηματική Ανασκόπηση και Μετα-ανάλυση Τυχαιοποιημένων Ελεγχόμενων Μελετών.

Περίληψη

Εισαγωγή: Το Σύνδρομο Ευερέθιστου Εντέρου (ΣΕΕ) αποτελεί μια συχνή διαταραχή του άξονα εγκέφαλος- έντερο χωρίς αποτελεσματική θεραπεία. Η εντερική δυσβίωση εμπλέκεται στη παθογένεση της νόσου και η μεταμόσχευση κοπράνων (ΜΚ) έχει εφαρμοστεί με σκοπό την τροποποίηση της μικροβιακής χλωρίδας. Τα αποτελέσματα των Τυχαιοποιημένων Ελεγχόμενων Μελετών(ΤΕΜ) είναι μέχρι τώρα αντικρουόμενα.

Μέθοδοι: Πραγματοποιήθηκε συστηματική ανασκόπηση της βιβλιογραφίας στις βάσεις μελετών PubMed, Embase, Medline, Cochrane, and ClinicalTrials.gov, και ΤΕΜ σε ενήλικους ασθενείς με ΣΕΕ που έλαβαν ΜΚ και παρακολουθήθηκαν για διάστημα τουλάχιστον 12 εβδομάδων συμπεριλήφθηκαν στη παρούσα μελέτη. Πρωτεύων καταληκτικό σημείο ορίστηκε η επίδραση της ΜΚ στη σοβαρότητα των συμπτωμάτων του ΣΕΕ.

Αποτελέσματα: Έγινε ανάλυση δεδομένων από την 12^{η} εβδομάδα από την παρέμβαση από 7 ΤΕΜς. Παρατηρήθηκε σημαντική ετερογένεια μεταξύ των μελετών (I^2 =87%). Το μοντέλο Random Effect (RE) χρησιμοποιήθηκε και ο λόγος αναλογιών OR υπολογίστηκε ως 1.9 με 95% διάστημα εμπιστοσύνης (CI) (0.56,6.5). Η υποομάδα φρέσκων/κατεψυγμένων-αποψυγμένων δείγματα μέσω ενδοσκόπησης ή ρινονηστιδικού σωλήνα (ΡΝΣ) είχε I^2: 71% και η ανάλυση με RE έδειξε OR: 3.97, 95% CI (1.35, 11.72).

Συμπεράσματα: Δε βρέθηκε στατιστικά σημαντική διαφορά στη σοβαρότητα των συμπτωμάτων στους ασθενείς που έλαβαν ΜΚ. Στην υποομάδα των ασθενών που έλαβαν φρέσκα/κατεψυγμένα- αποψυγμένα δείγματα μέσω ενδοσκόπησης/ΡΝΣ παρατηρήθηκε, ωστόσο, στατιστικά σημαντική διαφορά. Περαιτέρω έρευνα είναι αναγκαία προκειμενού να εξαχθούν ασφαλείς συστάσεις.

Λέξεις κλειδιά: σύνδρομο ευερέθιστου εντέρου, μεταμόσχευση κοπράνων

Introduction:

Irritable bowel syndrome (IBS) is a disorder of gut-brain interaction which is characterised by abdominal pain or discomfort in association with altered bowel habits, for at least 6 months. The diagnosis is a positive clinical diagnosis based on patient's symptoms, in the absence of 'red flag' symptoms and abnormalities on simple blood and stool tests.

The criteria used for the diagnosis and classification are Rome IV criteria, although in most of the studies analysed in this meta-analysis the earlier version (Rome III criteria) was used. The 4 types of IBS reported, are based on the morphology of bowel motions according to Bristol Scale and are the following: IBS-D (diarrhoea predominance), IBS-C (constipation predominance), IBS-M (mixed type of stools), IBS-U (unclassified).

IBS is a common disease, and the global prevalence was estimated 3.8% using ROME IV criteria to 9.2% using ROME III criteria, with a higher female prevalence (Vasant & et.al., 2021).

Although, not a lethal disease, the diagnosis of IBS caries a significant impact on social functioning, quality of life and ability to work. For instance, one in four patients would report sick -related leave. The annual direct and indirect cost of IBS is estimated up to 8 billion Euros in Europe and $10 billion in the USA (Vasant & et.al., 2021).

So far, there is no a single effective treatment for IBS.

Gut microbiota dysbiosis has been proposed as one of the key pathophysiological causes and therefore therapies altering the gut microbiota have been studied. As such, Faecal Microbiota Transplantation (FMT), defined as the transfer of screened and processed stools from highly selected healthy donor/donors to patients' gastrointestinal track, has been proposed as a direct way to 'correct' the microbiota imbalance which contributes to IBS. (Chong & et.al., 2019)

FMT has been proven to be effective and safe to treat recurrent Clostridium Difficile Infection (rCDI) (Goldenberg & et.al., 2021).

However, as regards IBS, the evidence of benefit based on the 7 published RCTs is controversial and heterogeneity in the involved studies was noted. The goal of the current review and meta-analysis is to further analyse the impact of FMT on IBS treatment.

Methods:

Meta-analysis was conducted according to the published Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement (Page & et.al., 2021).

Inclusion criteria entailed:

1) published prospective randomised control trials, double blinded, written in English language published up to September 2021

2) studies were conducted in adult patients (>16 years old) who were diagnosed with IBS according to a version of Rome Criteria (II, III or IV)

3) patients in the included studies could be classified in any of the IBS types

4) studies comparing FMT with control which could be either placebo or patient's own faeces (autologous)

5) studies had a sufficient follow up time (at least 12 weeks).

The primary outcome was the effect of the FMT in IBS severity-of-symptoms measured by OR. The significance of change in IBS symptoms as defined by each study was used and the OR was the expression of the probability of symptoms improvement after FMT/probability of symptoms improvement in control group. The 95% Confidence Intervals (95% CI) are provided.

Literature search in the PubMed, Embase, Medline, Cochrane, and ClinicalTrials.gov databases was performed.

References found in the identified articles as well as the previous relevant reviews were screened manually to minimise the risk for any missing studies.

Literature research was performed by a single researcher using the following terms: irritable bowel syndrome and faecal transplant or faecal transplantation or faecal microbiota transplant or faecal microbiota transplantation, irritable bowel syndrome and stool transplant or stool transplantation irritable bowel syndrome and FMT or bacteriotherapy or faecal implant.

1073 studies' titles were screened and duplicated and irrelevant titles were excluded. If title was relevant, then abstract was assessed. In 14 studies, the manuscript was further evaluated. Of those, 3 were excluded as proved to be post hoc analysis [(El-Salhy & et.al., 2021) (Goll & et.al., 2020) (Madsen & et.al., 2021)], 1 was excluded as only limited preliminary results were available in abstract form (Bruno & et.al., 2018), 1 was excluded as not RCT (Kumar & et.al., 2019) and 1 was excluded as limited data were available in abstract form. (Singh & et.al., 2019) Therefore, 7 studies were included in the meta-analysis.

The flowchart of the procedure followed is provided (Figure 1).

The exclusion of abstract results (Singh et all May 2019), due to limited data available, could have led to publication bias. Data were requested by contacting the authors, without success, though.

Whenever raw numbers were not given as such, they were calculated based on the provided percentages and the total sample size.

Tables 1 and summarise the main characteristics of studies included in the current analysis.

Data were analysed using RevMan version 5.4 software, Cochrane, London, UK ((RevMan)).

Metanalysis of the collected data was performed as well as subgroup analysis for identification of any possible significant covariant. The relevant forest plots were created to facilitate the visualisation of the data.

Random Effect (RE) was decided to be used when I^2>50%.

Analysis was based on week 12 post-intervention data. This was decided as 12 weeks is a landmark follow up period for most of the available RCT in the field.

All studies included were initially evaluated using the CASP checklist ((CASP), 2020), followed by the quality assessment of each study using the Jadad score (Jadad & et.al., 1996). The assessment was performed by a single researcher**.**

A funnel plot and Eager test were considered to assess for publication bias. However, since the number of included studies was small (<10 studies), these were not evaluated. Possible publication biases were the language limitation as well as the exclusion of the abstracts due to limited data entailed.

Results:

After the screening and study selection as described above, seven RCTs and a total of 461 patients were included in this meta-analysis.

Participants' characteristics, the characteristics of the RCTs included and those of the intervention used in each study are provided in Tables 1 and 2.

Three studies showed a statistically significant improvement of symptoms in patients who received FMT compared to control, three studies showed no statistically significant difference between the 2 groups (FMT vs control) and one study found statistically significant improvement of symptoms in favour of placebo. All data analysed were obtained at 12 weeks' time from the intervention.

Indeed, Johnsen et.al. showed a statistically significant improvement of symptoms. 36 out 55 patients of the FMT group vs 12 out of 28 of the control group reported improvement- a difference significant at the p=0.049 level (Johnsen & et.al., 2017).

Aroniadis et.al. implemented a crossover study, but only the first arm was used in this meta- analysis. There was no statistically significant difference with regards to the severity of symptoms between the two groups (improvement was noted in 11/22 patients that received FMT group and 14/23 that received placebo, p=0.46). This study was terminated early due to

an interim analysis that showed the lack of difference between the two groups (Aroniadis & et.al., 2019).

Halkjaer et.al. showed a statistically significant result in favour of placebo when comparing FMT vs capsule-non faecal containing- placebo given orally (8/22 vs 19/24 respectively with a p=0.008) (Halkjær & et.al., 2018).

Holster et.al. did not include in their study all types of IBS patients but only those found to have low amount of butyrate-producing bacteria in their faecal samples. Donors were selected to have high abundance of the butyryl-CoA CoA transferase gene in their faecal sample. The severity of symptoms was evaluated using the GSRS-IBS questionnaire. This study showed no statistically significant improvement (p=0.282) in the symptoms of FMT group (4/8) compared to the control group (1/8) (Holster & et.al., 2019).

El-Salhy et.al. included all types of IBS patients in their study. In this RTC patients were randomized to control vs 30g FMT vs 60g FMT via OGD, in a 1:1:1 allocation ratio. This study showed statistically significant improvement of symptoms in both FMT groups. For the group of 30g FMT, improvement was noticed 42/54 participants (vs 13/ 55 in control group) ($p<0.0001$) and for the group of 60g FMT in 49/55 participants (vs 13/ 55 in control group) ($p<0.0001$). For the purposes of this meta-analysis, groups of 30g FMT and 60g FMT were pooled together in one FMT group and were compared with the control group (El-Salhy & et.al., 2020).

Holvoet et.al. have included in their study patients with bloating and flatulence only, who had already failed 3 previous conventional treatments for IBS. This study concluded that there was a relief of general IBS symptoms as well as of abdominal bloating, and this result was statistically significant (improvement in 24/43 patients of the FMT vs 5/19 of the Control group) (p=0.03). Of note, the patients of this study were mainly male, the majority (94%) had tried low- FODMAP diet and only 11% were receiving psych medications (Holvoet, 2021).

The RCT of Lahtinen et.al was a crossover study, but only the first arm was included in this meta-analysis. Of note, three of the patients did not fulfil the Rome III criteria due to the fluctuating nature of their symptoms; however, it was decided to still be included in the study. A baseline characteristic analysis revealed the placebo group included more diarrhoea-predominant patients. The results showed no significant difference in the improvement of symptoms between the two groups (improvement in 11/23 participants of the FMT group vs 11/24 of the control group) (p=0.690) (Lahtinen & et.al., 2020).

It should be noted that all studies were double blinded randomised control trials and was unlikely for the blinding to be broken. Randomisation was properly performed and no significant loss of participants in follow up was identified in any of the studies. In case of patient loss in follow-up, this was reported, and sufficient explanation was provided.

All studies used the Rome III criteria for the diagnosis of IBS, except for El-Salhy et.al. that used Rome IV criteria. IBS SSS score was used by all studies apart from Holster et.al. who used GSRS-IBD score and Holvoet et.al. who used a dichotomous question.

Of note, the patients included in the various studies had heterogeneous characteristics such as different types of IBS, had previously trialled and failed in variable treatments and/or had specific bowel microbiota characteristics- all of which were defined by the inclusion criteria of each study. (Table 2)

The donors' number and characteristics as well as the sample preparation for the FMT and the route of administration also differ among the several studies (Table 2).

A meta-analysis was performed to evaluate, in balance, the impact of FMT on the severity of IBS symptoms.

As shown in Figure 2, I^2= 87%, which suggests that 87% of the variability in the FMT effect estimate is due to real differences and only 13% due to chance, indicating high

heterogeneity between the studies included in the current meta-analysis, bearing in mind though, that the number of studies included is small.

Therefore, the RE model was decided to be used to calculate the pooled estimate. The OR was calculated as 1.90 with CI (0.56,6.50). In other words, FMT appears to benefit patients with IBS but its effect is not a statistically significant result as 1 is included in the CI 95%. (Figure 2)

To deal with heterogeneity in between the studies included, a subgroup analysis was performed with the following subgroups:

- First subgroup analysis was Endoscopy & fresh/frozen FMT vs oral & capsule.

In this subgroup analysis, FMT through NJ tube was included in the endoscopy group. Although strictly speaking FMT through NJ tube is not an endoscopical procedure, it still bypasses the stomach and the duodenum mimicking technically the FMT through OGD. The 'endoscopy fresh/frozen FMT' group has still a high heterogenity in between the studies included with the I^2 equal to 77%. The OR in this group was 3.97 with CI 95%: (1.35,11.72), in the RE analysis -which represents a statistically significant relation. In the 'oral & capsule' group, heterogeneity was high as well (I^2=61%). However, the OR was 0.32 with CI 95%: (0.08,1.33). The Chi Square test for subgroup differences equals to 7.63 with p=0.006, which indicates a statistically significant difference between the 2 subgroups as regards the impact of FMT, with high heterogeneity though (I^2=86.9%) (Figure 3).

- Second subgroup analysis was 'IBS-C included' versus 'IBS- C excluded' group of studies

The 'IBS- C included' group had a great heterogenity (I^2=95%) and with RE effect there was no statistically significant difference in symptom improvement (OR=2.53 CI 95%: (0.08, 75,88)). In the 'IBS-C excluded subgroup' the level of heterogenity was lower (I^2=44%) but again, there was no statistically significant difference in symptom improvement (OR=1.62,

CI 95%: (0.78,3.39)). There was no statistically significant difference between the 2 groups as regards the impact of FMT (p=0.8) (Figure 4).

Discussion:

Irritable bowel syndrome is a common disease with no identified effective conservative treatment so far. The aetiology is considered to be multifactorial, and the pathophysiology is yet unclear (Hadjivasilis & et.al., 2019). Gut microbiota dysbiosis is one of the proposed pathophysiology mechanisms and therefore faecal transplantation could play a role in changing the microflora towards a healthier microbiota profile that would improve patients' symptoms (Chong & et.al., 2019). So far, the available RCTs in this field have had controversial outcomes and all researchers agree that more evidence is required to form a strong recommendation. In this meta-analysis data, published in 7 randomised control trials, implemented between 2017 and 2020, were analysed. On balance, it appears that the FMT might have a beneficial impact on IBS symptoms. However, there is great heterogeneity in between studies and the relation is not statistically significant. Subgroup analysis showed that transplanting fresh or frozen donor material via colonoscopy, NJ tube or OGD showed statistically a significant improvement in IBS symptoms with high study heterogeneity, though.

The limitations of this meta-analysis include the small number of studies assessed and the possibility of excluding studies published in other languages than English. Also, the data analysis was performed by a single researcher. Furthermore, the study population is patients

from north-central Europe and USA and therefore results might not be applicable to patients of different origins (Gwee & et.al., 2018). Further research is needed to validate any possible impact of FMT on populations of different origins.

Also, all data analysed in the present study were collected at 12 weeks post intervention. There is a lot of evolving research on the appropriate follow-up time and more studies are required to conclude on the possible long term FMT impact on symptoms improvement/microbiota change. Furthermore, increasing the initial FMT dose or re-transplantation has been proven effective, when patients did not respond to the first FMT (El-Salhy & et.al., 2019).

Another meta-analysis limitation is the heterogeneity of the studied population. More specifically, in some of the studies only IBS-D patients were included. This is important as IBS subtypes have been considered to have different pathophysiological mechanisms and has previously suggested that the responsible microbiota varies amongst IBS subtypes (Wang & et.al., 2019). Nevertheless, the subgroup analysis performed by our group in the small number of studies available has not shown any significant difference between studies containing only IBS-D, IBS-M and those with all IBS subtypes.

Similarly, in one study (Holster & et.al., 2019) the additional criterion of patients with a low amount of butyrate-producing bacteria in their faecal samples was applied. In other studies, failure to previous treatment was a prerequisite ((Aroniadis & et.al., 2019), (El-Salhy & et.al., Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study, 2020) (Holvoet, 2021))

Lastly, the population recruited in this study was diagnosed based on the earlier Rome III criteria (except for the study by El-Salhy et.al., 2019), and not the most updated Rome IV criteria which are more restrictive and might have excluded some of the patients included in this meta- analysis (Vasant & et.al., 2021).

As regards the included studies design, it must be noted that different questionnaires were used to evaluate the severity of symptoms. Indeed, most of the studies used the IBS SSS (Francis & et.al., 2003) but Holister et.al uses GSRS-IBS and Holvoet et.al. uses a dichotomous question to assess the response to FMT (Wiklund & et.al., 2003). Furthermore, even amongst the 5 studies using the IBS SSS, Johnsen et.al. has defined as symptom relief a reduction more than 75 points, while the rest have used 50 points reduction as a hallmark. This could mean that the possible effect in FMT can be underestimated or overestimated depending on the standard taken each time.

Of note, the criteria for donors' selection were, also, not unified among the studies. All studies have chosen healthy, young adults without a history of metabolic or malignant disease and precautions were taken to minimise transmission of infectious diseases by screening the faeces and the blood of the patients. In the study of El- Salhy et. al., the donor had to be vaginally delivered and breastfed, while in the study of Halkjaer per vaginal delivery was also in the criteria. In the study of Holister et.al., the donors had a high abundance of the butyryl-CoA CoA transferase gene in their faeces.

The number of the donors in the studies included also varied. Some, also, used pooled faeces microbiota from more than one donor. Unfortunately, the small number of RTCs did not allow further subgroup analysis with regards to this characteristic.

The method of processing the faecal sample also differs amongst the studies, as these could be fresh, frozen and thawed or frozen capsules. The route of the faecal microbiota transplantation varied, too. The possible routes included per os, via colonoscopy, OGD or NJ tube. A subgroup analysis was performed. Administration by NJ tube was grouped with the OGD as the former bypasses the gastro and duodenum and places the FMT lower in the Upper GI avoiding the exposure to stomach acid. Patients who received fresh/frozen and thawed FMT also had the transplantation installed through colonoscopy/OGD/NJ and

therefore they formed the same subgroup named as 'Endoscopy &fresh/frozen'. On the other hand, all patients who received capsules had the FMT given orally and therefore those patients formed the group 'Oral& Capsule'. The subgroup analysis revealed significant improvement in patients in the 'Endoscopy& Fresh/Frozen' group. However, this effect cannot be safely attributed neither to the process of the samples nor the site the FMT was installed. Of note, in a previous meta- analysis of 4 of the above studies, FMT via colonoscopy or NJ was found to be superior to autologous transplantation in subgroup analysis (Xu & et.al., 2019).

The faecal content dose varied among the studies, too, between 30-80g for fresh/frozen and thawed FMT and 12g daily for 6 days to 28g administered over 3 consecutive days when given orally. There might be a dose related efficacy of FMT, as in a study (El- Salhy & et.al., 2019) increasing the dose was one of the ways to achieve response to previously non responders to FMT. Thus, more research is needed to identify the most effective dose.

Furthermore, all studies except 2 ((Aroniadis & et.al., 2019), (El-Salhy & et.al., Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study, 2020)) have used bowel cleansing before FMT. This is important to note as bowel cleansing has been found to change the bowel microbiota at least transiently. However, the effect that this might have on faecal transplant is yet to be clarified (Drago & et.al., 2019).

Moreover, glycerol was used in processing faecal samples in the studies of (Johnsen & et.al., 2017), (Halkjær & et.al., 2018) (Holster & et.al., 2019) but this is also used for the IBS-C treatment. Therefore, if this itself had an impact on the IBS symptoms is yet unknown.

Also, all studies considered in the current meta-analysis except of this of El-Salhy et.al. had less than 100 participants, and that of Holster et.al. had only 14 participants. This has negative effect on the power of these studies.

Furthermore, co-founding factors were not thoroughly addressed and investigated in the studies included.

More specifically, diet is known to affect the severity of symptoms in IBS. (Benech & et.al., 2020). However, this is only addressed in the studies of Johnsen et.al. and Holster et.al. in which participants were asked to maintain their diet and to keep a food diary. Therefore, it is unclear whether dietary modifications could have contribute to the changes in IBS symptoms severity noted in the rest of the studies.

In addition, previous research work has suggested that Post-Infectious IBS (PI-IBS) have particular patterns of bacterial abundance (Downs & et.al., 2017). In the study of Aroniadis et.al. (Aroniadis & et.al., 2019), researchers make the hypothesis that FMT might better act in PI-IBS and therefore have performed a post hoc analysis to test that hypothesis. Indeed, patients with PI-IBS had a better symptom improvement, but this was not statistically significant in the small number of analysed patients. Holister et.al. (Holster & et.al., 2019) have also reported the number of patients with PI- IBS included in their study. However, the rest of the studies have not considered this possible confounding factor.

It is, also, important to mention that all studies except for the study of Johnsen et.al. have performed a microbiota analysis in the study population. Several of these studies report a change in microbiota after FMT such as reporting richer or more direct microbiota or change towards the donor microbiota. In some of the studies, these changes were correlated with significant improvement of IBS symptoms (El-Salhy & et.al., 2020) (Holvoet, 2021) (Goll & et.al., 2020) while in others not (Halkjær & et.al., 2018) (Lahtinen & et.al., 2020). These data though, is hard to group and compare as each study uses a different method of evaluating the baseline microbiota as well as any change. Further studies are needed to understand these changes and correlate them with any clinical improvement.

As regards the background characteristics heterogeneity, sex is probably the most important to mention as in the study of Holvoet et.al. female participants were found to have a significantly better response to FMT. A post hoc analysis (El-Salhy & et.al., 2021), though, based on sex failed to reveal any statistically significant relationship effect of sex on the success of FMT in patients with IBS. However, in particular females with IBS-D responded better and had more significant reduction of symptoms than males after FMT.

As regards safety, FMT is considered to be a safe procedure as no severe adverse events such as deaths or severe morbidity were reported in the analysed RCTs. Some of the adverse events reported were mostly attributed to the endoscopic procedure used rather than the FMT itself e.g. transient nausea and vertigo. Recent concerns were raised, though, based on 6 cases in which patients developed enteropathogenic Escherichia Coli and Shigatoxin-producing Escherichia Coli infection, after treated for rCDI- 2 of whom eventually died (Camilleri, 2020). However, it is reasonable to wonder whether these were high-risk cases as FMT were performed immunocompromised patients who are susceptible to infections (El-Salhy M. e., 2021). The safety of FMT is even more relevant nowadays; given COVID-19 infection could be transmitted with FMT with detrimental consequences (Ianiro & et.al., 2020).

In the strengths of this meta- analysis, it should be noted that it includes 7 RCTs- more than the previous meta-analysis published on the same subject (Myneedu & et.al., 2019) (Xu & et.al., 2019).

This study hopes to contribute towards a better understanding of the role of FMT in the treatment of FMT as well as the selection of 'right' donors and 'right' patients so that FMT would be more effective. As several trials are scheduled or just completed, there is hope that more evidence would be available soon (Goldenberg & et.al., 2021).

Conclusions

In conclusion, we have conducted a meta-analysis assessing the effect of FMT on IBS symptoms based on RCT data 3 months post intervention. FMT might have a role in the treatment of IBS as our study showed that it improves IBS symptoms severity, but this was not a statistically significant result. Subgroup analysis revealed that transplantation of fresh or frozen faecal microbiota directly into the bowel with colonoscopy, NJ or OGD showed a statistically significant improvement of IBS symptoms, though. However, it should be stressed that the Random Effect model was used in the present meta-analysis, as high heterogeneity amongst the included studies was noted. Thus, further research is required to identify the 'best' patients, and the 'right' way as well as 'right' timing for the faecal transplantation to maximise its potential effect on the IBS symptoms' severity.

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Abbreviations:

RCT: Randomised Control Trial
rCDI: recurrent Clostridium Difficile Infection
RE: Random Effect
FE: Fixed Effect
CI: Confidence intervals
OR: Odds Ratio
IBS: Irritable Bowel Syndrome
IBS-D: irritable bowel syndrome- constipation
IBS-C: irritable bowel syndrome- diarrhoea
IBS-M: irritable bowel syndrome- mixed type
IBS-U: irritable bowel syndrome- unclassified
FMT: faecal microbiota transplantation
OGD: Oesophago-Gastro-Duodenoscopy
Colono: colonoscopy
NJ tube: NasoJejunal tube
FODMAP: fermentable oligo-, di-, mono-saccharides and polyols
IBS SSS: irritable bowel syndrome severity scoring system
PI-IBS: post infectious irritable bowel syndrome

Figures and Tables:

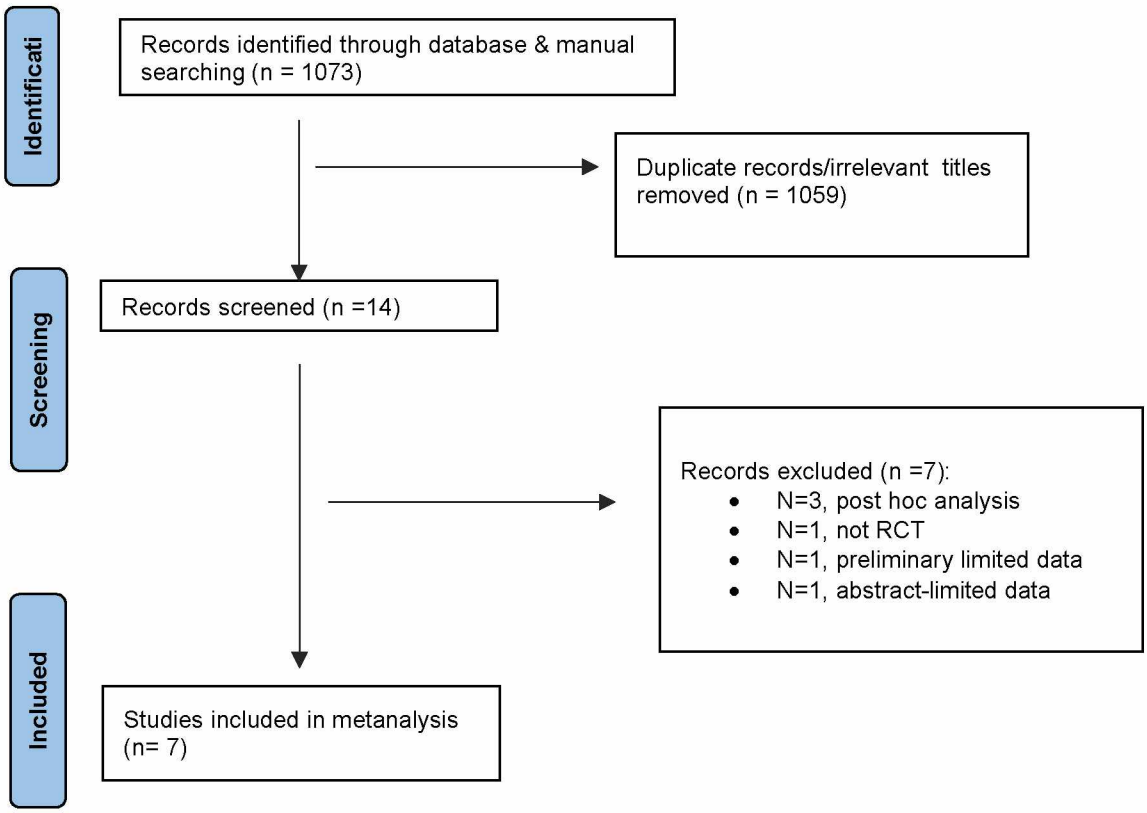

Figure 1. Flowchart of literature review

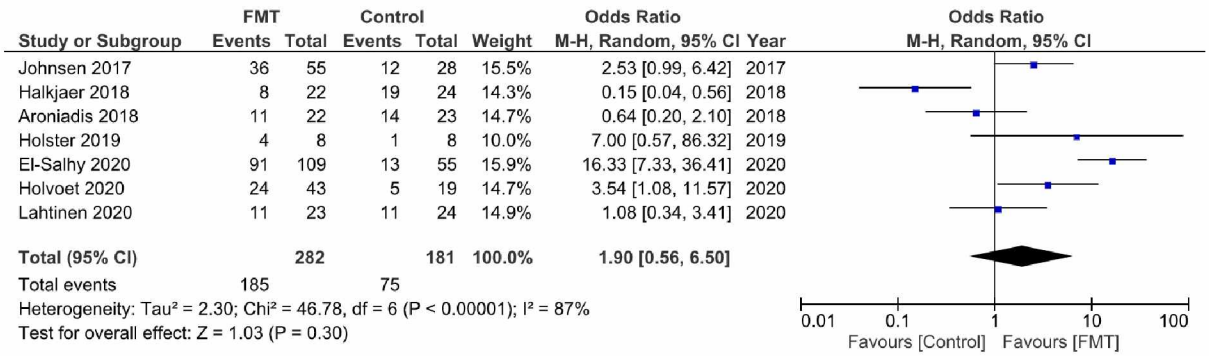

Figure 2a. Forest plot on meta-analysis on the impact of FMT on IBS related symptoms improvement

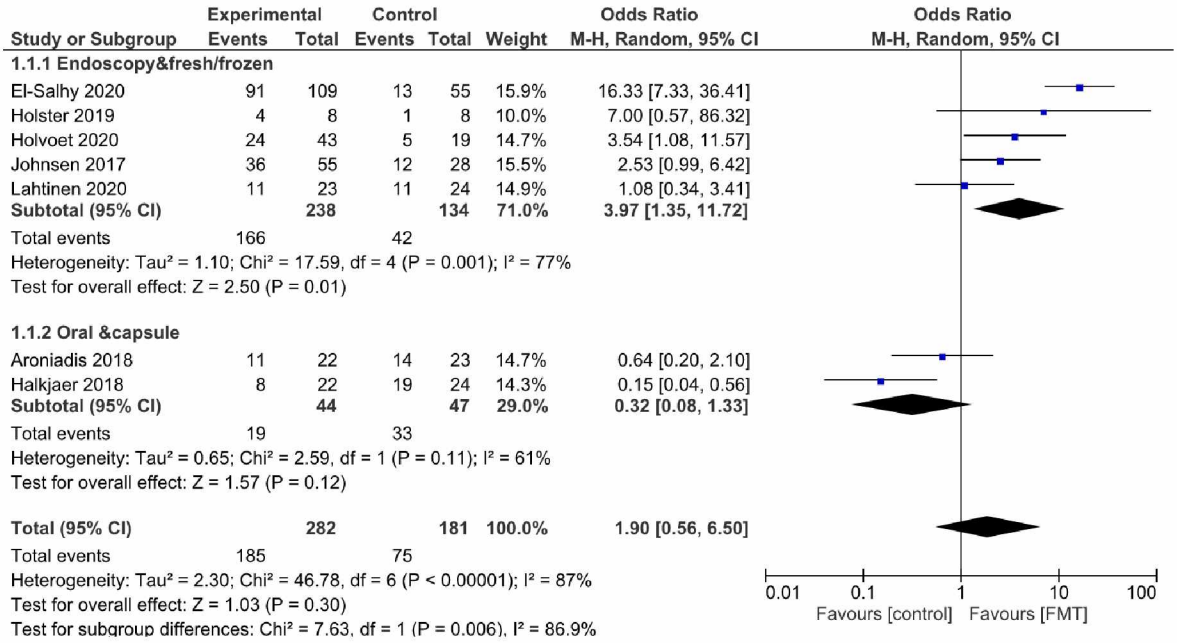

Figure 3: Forest plots of subgroup analysis of 'endoscopy & fresh/frozen vs oral & capsule

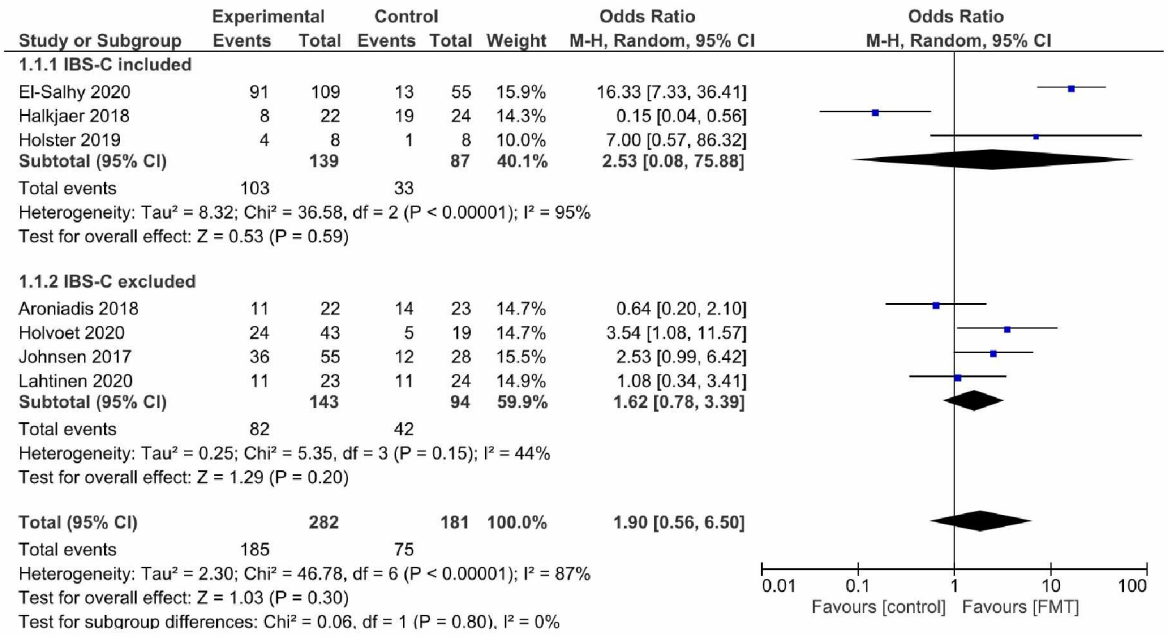

Figure 4: Forest plots of subgroup analysis on 'IBS-C included' vs 'IBS-C excluded' group

Table 1: Study Characteristics

Study	Type of study	Country	size	Questionnaire	Primary outcome	IBS diagnosis criteria	Result	F/U (mo)
Johnsen 2017	Single	Norway	83	IBS SSS	Symptom relief of more than 75 points assessed by IBS-SSS, 3 months after FMT	Rome III	Statistically significant symptom improvement	12
Aroniadis 2018	Multi &cross over trial	USA	45	IBS SSS	The difference in disease severity between the FMT group and the placebo group at 12 weeks, as measured by IBS-SSS recorded at individuals' sites, with the IBS-SSS scores then compared at a central location.	Rome III	No statistically significant symptom improvement	6
Halkjaer 2018	Single	Denmark	46	IBS SSS	To evaluate the reduction of IBS-SSS in the treatment group compared with the placebo group at 3 months	Rome III	Significant change in symptoms favouring placebo	6
Holster 2019	Single	Sweden	14	GSRS-IBS	The effect of FMT on IBS symptoms using the IBS version of the GSRS-IBS	Rome III	No statistically significant symptom improvement	6
El-Salhy 2019	Sigle &parallel group	Norway	164	IBS SSS	The primary endpoint was a reduction in the IBS-SSS total score of ≥50 points at 3 months following transplantation	Rome IV	Statistically significant symptom improvement	3
Holvoet 2020	Single &pilot crossover	Sweden	61	Dichotomous question	The primary endpoint of this pilot study was the relief of general IBS symptoms and abdominal bloating at 12 weeks following the FMT	Rome III	Statistically significant improvement in both end point questions	12
Lahtinen 2020	multi	Finland	49	IBS SSS	A sustained relief of IBS symptoms throughout the 52-week follow-up period	Rome III	No statistically significant symptom improvement	12

Table 2: Characteristics of study population and intervention applied

study	Sex F/ M	Mean Age	IBD Severity	IBS classification	Patient preparation	Grams of faecal content	Number Of Donors	ROA	Type of Intervention	Control
Johnsen 2017	55/ 28	44.5	Moderate to severe	IBS D:32 IBS M:39	Loperamide 8mg, bowel preparation	50-80g	2, mixed	colono	Fresh or frozen-thawed	Own faeces
Aroniadis 2018	18/ 30	37.5	Moderate to severe & refractory	IBS D:45	PPI	28g	4, not mixed	orally	Frozen capsule	Non faecal placebo
Halkjaer 2018	30/ 16	36.4	Moderate to severe	IBS D:15 IBS C:17 IBS M:19	Fasting one hour post	12g daily	4, mixed	orally	Frozen Capsule	Non faecal placebo
Holster 2019	8/8	36.5	N/A	IBS D:9 IBS C:4 IBS M:3	Loperamide 4mg & Bowel preparation	30g	2, not mixed	colono	Frozen	Own faeces
El-Salhy 2020	133 /31	40.1	Moderate to severe - refractory to FOD MAP	IBS D:63 IBS C:62 IBS M:39	N/A	30g or 60g	1	OGD	Frozen-thawed	Own faeces
Holvoet 2020	38/ 24	38	Severe abdominal Bloating & refractory	N/A (IBS D &IBS M)	Macrogol	n/a	2, not mixed	NJ tube	fresh	Own faeces
Lahtinen 2020	29/ 20	46.3	N/A	IBS D:25 IBS M:7 IBS U:14 Other:3	bowel preparation	30g	1	colono	Frozen - thawed	Own faeces