*Answering reviewer 02531171*

Thank you very much for giving us such valuable suggestions. We have made revisions or explanation point by point.

General comments:

carefully check the entire manuscript for consistent formatting, typos, inappropriate use of capitalisation, use of brackets and grammatical errors.

We have carefully checked and corrected the errors.

Specific comments:

Abstract: This needs to be re-written. Provide context to justify the investigation. Less focus on the IBS symptoms in the results section – focus on the novel findings. Reporting all of the results makes the abstract difficult to read and the conclusions difficult to interpret.

This abstract has been re-written. Study background has been added and the results section has been simplified on P 3 of the manuscript.

Methodology: spell out acronyms on first mention (e.g. H&E).

It has been revised on line 10, P7 and line 2, P9.

Provide additional details on the biopsies – mucosal pinch biopsies or full thickness samples?

It has been revised on line 18, P3 and line 7, P7.

Provide details on the power calculations used to select group sizes.

Based on a previous research [1] which focused on BDNF in IBS patients, we calculated the minimum sample size using the software PASS to make the difference of BDNF levels significant between the two groups. The output of the calculation was as below. The sample size in our study was larger than the calculated minimum sample size.

**Two-Sample T-Tests Allowing Unequal Variance**

**Numeric Results for Two-Sample T-Test Allowing Unequal Variance**

Alternative Hypothesis: H1: δ = μ1 - μ2 ≠ 0

| Target Power | Actual Power | N1 | N2 | N | Target R | Actual R | μ1 | μ2 | δ | σ1 | σ2 | Alpha |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.90 | 0.91376 | 4 | 6 | 10 | 1.50 | 1.50 | 1.0 | 2.0 | -1.0 | 0.3 | 0.5 | 0.050 |

**References**

Results: This group is unusual with a male prevalence (22 vs 9). This should be commented upon.

It has been interpreted in the demographic section of the discussion on P 13.

Age is trending towards being different between patients and controls (similar p value to that of depression, which was commented upon).

It has been corrected in the psychological disorder section of the discussion on P 13.

Should 'media' pain be 'median'?
It has been corrected on line 15, P 11.

IBD-D should be IBS-D – check carefully throughout.
We have checked carefully and corrected the errors like this.

Include p values, or symbol of significance on the histogram in figures 2 & 3.
P values have been added to the figures.

Discussion: The discussion needs significant additional work – some specific comments below.
Given that afferents innervating the gut terminate below the mucosa, why did the authors choose to examine neural levels from mucosal biopsies?
The plexuses of the enteric nervous system are located in the muscle, the submucosa and the lamina propria of the mucosa of humans [2]. Mucosal pinch biopsy contains mucosa and part of submucosa. Some biopsies contains large part of submucosa while some contains small part of submocosa. So we only examined the nerve fibers in the lamina propria to avoid the bias of the sits of sampling. And that has been explained on line 8-11, P 15 of the manuscript.

That authors state that 'BDNF may have an effect on the intestinal neuronal plasticity in IBS-D patients', what neurons are you referring to?
It has been reported that BDNF has an effect on the growth of both sensory and motor neurons [3]. In this study, we referred to the sensory neurons, for that we evaluated the abdominal pain severity and visceral sensitivity of IBS-D patients and the two parameters were both correlated with the level of BDNF. It has been revised on line 8, P 13 of the manuscript.

What is the likely source of mucosal BDNF?
Several studies demonstrated that the potential source of intestinal mucosal BDNF were the epithelial cells in mice, pigs and humans [4-6]. In our study, the BDNF immunoreactions were mainly located in the epithelial cells and the lamina propria, which was consistent with the previous researches. This has been added on line 29, P15.

The authors should comment on whether there is validity in the findings given that the cohort is not representative in terms of gender.
In all IBS patients, females may account for the majority. But, females were more predisposed to exhibit the constipation-predominant subtype, while IBS-D subtype was less common in women [7]. The gender ratio in our study was similar to that of Yang's [8] and Xu's [9]. As a result, the cohort was representative of IBS-D patients.

Please provide further explanation/context for the phrase 'Furthermore, males often had more trust in this study than did females.'
It has been explained after the sentence on line 16, P 13.

The authors comment that 'was not statistically significant, possibly due to the relatively small sample size'; was the study under-powered?
As we had calculated the sample size, the sample size in this study was enough to compare the main parameters such as BDNF levels and visceral sensitivity of the IBS-D group and the control group. The sample size was ok in whole. The difference on depression score between the groups was not significant in this study. And this sentence has been corrected on line 23, P 13.

The authors state 'Furthermore, we also noticed that IBS-D patients usually complained more about abdominal pain when they were taking colonoscopy tests, consistent with visceral hypersensitivity.' This is not an objective measurement. Please provide data or remove this sentence.
This sentence has been deleted.

Please relate the statement 'Besides, neuromediators (such as substance P, calcitonin gene-related peptide (CGRP) and serotonin), mucosal inflammation and the intestinal microbiota also have effects on visceral hypersensitivity [36, 38].' to the study findings.
This sentence was stated for that we wanted to explain the possible mechanism of visceral hypersensitivity.

Rather than simply summarising the methods and findings relating to the BDNF experiments, discuss them in the context of the literature.
We have deleted some description of the methods and findings and added discussion relating BDNF experiments on P 15.

In agreement with the comments of the authors on the limitations of the study, I think and PI-IBS patients should be identified, and potentially excluded from this study.
Due to the limited time and conditions and the strict criteria of Rome IV (the prevalence of the IBS patients according to Rome IV only was half of that according to Rome III [10].), the sample size in our study was relatively small. So we did not study them separately. But we have compared the main parameters, such as BDNF and visceral sensitivity, of PI-IBS and non PI-IBS subgroups. We found no significant difference between the two subgroups. Considering the relatively small group size of the IBS-D group, we did not exclude the PI-IBS group. But it would be the next step of our research team to study them separately.

*Answering reviewer 02155135*

The article is interesting. Moreover I suggest to investigate also another factor implicated in the IBS-D to better understand the effects of BDNF. I suggest making the different parts in the abstract more similar by length. The discussion is very long and it is difficult to read. It is important a revision of grammatical errors in the document.

Thank you very much for your advice of investigating another factor implicated in the IBS-D and it is very useful for us to make the article better. However, we have no samples remained, which is a pity. We have made a revision on the abstract and the words number of each section was up to standard of *The Guidelines for Manuscript Preparation and Submission*. The discussion has been simplified. Grammatical errors was checked thoroughly and corrected.

# References:


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3 Barde Y, Tucker KL, Meyer M. Neurotrophins are required for nerve growth during development. *Nat Neurosci* 2001; 4: 29-37 [DOI: 10.1038/82868]

4 Yu YB, Zhao DY, Qi QQ, Long X, Li X, Chen FX, Zuo XL. BDNF modulates intestinal barrier integrity through regulating the expression of tight junction proteins. *Neurogastroenterol Motil* 2017; 29: [DOI: 10.1111/nmo.12967]

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6 Boesmans W, Gomes P, Janssens J, Tack J, Vanden BP. Brain-derived neurotrophic factor amplifies neurotransmitter responses and promotes synaptic communication in the enteric nervous system. *Gut* 2008; 57: 314-322 [DOI: 10.1136/gut.2007.131839]

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10 Bai T, Xia J, Jiang Y, Cao H, Zhao Y, Zhang L, Wang H, Song J, Hou X. Comparison of the Rome IV and Rome III criteria for IBS diagnosis: A cross-sectional survey. *J Gastroen Hepatol* 2017; 32: 1018-1025 [DOI: 10.1111/jgh.13642]