Thank you for your comments on our manuscript. Point-by-point responses to reviewer comments are found below.

Reviewer #1:

Was the weight recorded before or after the onset of diarrhea? If before, how to avoid recall bias?

BMI was based on current height and weight. However, we asked patients if they lost weight following the onset of diarrhea. As noted in the abstract and elsewhere, patients with microscopic colitis (MC) reported more weight loss after the onset of diarrhea. To examine whether this weight loss could explain the association between BMI and microscopic colitis we performed a stratified analysis. As shown in Table 2, the association between BMI and microscopic colitis was similar among the group of patients who did not lose weight. In other words, weight loss due to the disease does not explain the strong inverse association between BMI and microscopic colitis.

For recall bias to occur, the reporting of weight and height would have to be differential between the microscopic colitis cases and the control group. Because both groups of patients were referred for colonoscopy for diarrhea we think that it is unlikely that any bias would occur.

> Page 10. Recall of past exposures may be inaccurate, but we would not expect the recall for cases and controls to be differential as all of the patients were enrolled in the study because of diarrhea.

Previous studies have shown that proton pump inhibitors (PPIs) and non-steroidal anti-inflammatory drugs (NSAIDs) are associated with microscopic colitis. Why were they not analyzed as confounding factors in this study?

We have previously reported that PPIs and NSAIDS are not associated with microscopic colitis in this population.[1] Because these drugs were not associated with microscopic colitis they could not confound the results.

Table 1: It does not make sense to include the standard deviation (SD) of age in the "Percent" column.

We have added (SD) in the age row to more clearly indicate that number represents a standard deviation and not a percent.

Reviewer #2:

The definition of diagnosis of microscopic colitis is critical. Classically, colonic biopsy shows histological features: > 20 intraepithelial lymphocytes per 100 epithelial cells in lymphocytic colitis (LC) and 10-20 µm of a thickened subepithelial collagen band in collagenous colitis (CC). Would you describe more precisely the pathological view of the diagnosis of this study?

The diagnosis of microscopic colitis in this study was made by a single experienced GI pathologist. The diagnosis of lymphocytic colitis was based on an increased number of

intraepithelial lymphocytes. Additional features of MC included surface epithelial damage and increased lamina propria chronic inflammation, with minimal crypt distortion or active cryptitis. Collagenous colitis was defined by deposition of subepithelial collagen forming a band > 10 µm thick. In the present study we excluded patients with indeterminate microscopic colitis defined as a sparse number of lymphocytes.

MC is relatively easy to recognize pathologically. Both interobserver and intraobserver agreement have been found to be excellent with mean intraobserver agreement with the final diagnostic category of microscopic colitis vs. non-microscopic colitis of 95%.[2]

In our study, we recorded the findings of the clinical pathologist and the research pathologist. When there was a disagreement between the study pathologist and the clinical pathologist, the slides were re-read by the research pathologist. The diagnosis was based on the final reading of the study pathologist. A 20% sample of H&E stained study slides were re-submitted to the study pathologist to evaluate reliability. The agreement was 100% - the second independent reading agreed with the first 100% of the time.

We have made the following changes in the manuscript on page 4.

> Cases were patients with microscopic colitis on biopsy defined by increased number of intraepithelial lymphocytes. Additional features included increased lamina propria chronic inflammation, with minimal crypt distortion or active cryptitis. Collagenous colitis was defined by a thickened subepithelial collagen band. Slides were initially reviewed by a clinical pathologist. The slides were then re-read by the study pathologist. When there was a disagreement between the clinical pathologist and the research pathologist, the research pathologist re-read the slides. In addition, a 20% sample of slides were resubmitted to the research pathologist. After excluding indeterminate colitis, there was a 100% match between the initial and final reading by the research pathologist.

The prevalence of MC differs from Western countries and others. Concerning MC, many discrepancies exist between nations. The authors should describe this issue in the discussion.

We have made the following changes to the discussion, page 9

> The study was conducted in a developed country. Geographic variations in the incidence of microscopic colitis have been reported but there have been a limited number of direct comparative studies. [3] There are few studies from developing contries.[4]

Originally, the endoscopic findings of patients with MC have been described as normal; however, recent reports described endoscopic abnormalities such as changes in color, vascular pattern, changes in surface property, and mucosal tears (linear ulcers/scars, "cat scratch," crack-like grooves, etc. The authors should described the endoscopic pattern of this study.

While the mucosa in microscopic colitis was previously reported to be normal, it is increasingly recognized that abnormalities are present in nearly 40% of patients.[3] We did not record subtle endoscopic findings in this study. We have made the following changes in the manuscript.

> Page 3. It is increasingly recognized that endoscopically visible lesions can be recognized in nearly 40% of patients although they are non-specific.[3]
>
> Page 4. Patients with signs of gross inflammation on colonoscopy were excluded. Patients with subtle or isolated mucosal abnormalities were not excluded.

They did not describe the treatment of MC (discontinuation of the suspected drug, steroids, Biologics, etc.) and followed up enough.

The purpose of this study was to examine risk factors for the development of microscopic colitis. Treatment and outcomes are beyond the scope of the study.

The weakest point of this study was the too-small number of patients in single-center experience who could not fully explain the evidence of the etiology of MC.

We recognize and acknowledge the small size of the study. In fact, the manuscript states (page 10) "A limitation of the study was the small size, particularly for men. Microscopic colitis is an uncommon disease and most reports in the literature are hampered by small numbers." However, the study was large enough to detect strong and statistically significant differences between the cases and the controls with respect to BMI and birth control pills. If the study were "too small" there would be no significant findings

Reviewer #3:

The article is a case-control study, and the majority of cases are women, and it is recorded retrospectively in the form of questionnaire, which may produce bias.

We acknowledge that a retrospective study is susceptible to recall bias, but as noted in our response to reviewer 1, bias only occurs when there is differential recall by the cases and the controls. We designed our study to reduce the risk of recall bias by enrolling cases and controls with chronic diarrhea with the diagnosis unknown at the time of enrollment. As is currently stated in the manuscript, page 10

> Recall of past exposures may be inaccurate, but we would not expect the recall for cases and controls to be differential as all of the patients were enrolled in the study because of diarrhea.

There is no information on the type of oral contraceptives or the type or dose of postmenopausal hormones used, nor the detection results of case-related hormone levels, nor the analysis results of gut microbiota. It cannot be inferred that the microscopic pathogenesis of colitis may involve the hormonal effect of obesity or gut microbiota

We agree that there was no information on the type of oral contraceptives or the dose of postmenopausal hormones that were used. This was acknowledged on page 10 among the limitations of the study. We hope that our findings will lead to additional research by us and by others to explore hormones in more detail.

We agree that it cannot be inferred that the pathogenesis of colitis involves hormonal effects or the gut microbiome. In the conclusion of the abstract we state: “Mechanisms are unknown but could involve hormonal effects of obesity or the gut microbiome.” The purpose of that statement was simply to speculate about possible biological mechanisms and to motivate additional research to understand the findings.

Suggestion: carefully revise the discussion part to fully explore the relationship between microscopic colitis and body weight, gender, oral contraceptives, etc.

The discussion includes a full page that reviews the world literature on the association between BMI and microscopic colitis. As noted in the discussion, the literature is quite limited. In addition, there are several paragraphs that consider whether the findings could be confounded. For example, smokers have lower BMI and greater risk for MC. However, in our study, smoking was not a risk factor for MC. We examined whether the choice of controls might have explained the major findings by conducting stratified analysis of the IBS patients. The IBS group did not explain the findings. We also included a paragraph discussing the lower risk for disease among men, and speculating whether androgens might be related. We included 3 paragraphs – almost one page - in the discussion exploring the association of hormones with microscopic colitis.

References

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2. Limsui D, Pardi DS, Smyrk TC, et al. Observer variability in the histologic diagnosis of microscopic colitis. Inflamm Bowel Dis 2009;15:35-8.
3. Miehlke S, Guagnozzi D, Zabana Y, et al. European guidelines on microscopic colitis: United European Gastroenterology and European Microscopic Colitis Group statements and recommendations. United European Gastroenterol J 2021.
4. Gado AS, Ebeid BA, El Hindawi AA, et al. Prevalence of microscopic colitis in patients with chronic diarrhea in Egypt: a single-center study. Saudi J Gastroenterol 2011;17:383-6.