May 22nd, 2022

Dear Dr. Tarnawski,

Thank you for considering our study, "*Prevalence and Factors Associated with Vitamin C Deficiency in Inflammatory Bowel Disease*", for conditional acceptance in *World Journal of Gastroenterology*. We appreciate the feedback that we received from the peer review. Below, please find our point-by-point response to each of the comments raised in the peer review. Attached, you will also find a revised version of the manuscript. We also created a version with highlighted revisions that corresponds with the page numbers and paragraphs in the document below; we are happy to provide this, if you would like. We hope this revision is to your satisfaction.

Sincerely,

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Reviewer #1:

Comment:

There being limited data available on deficiency of micro nutrients like Vitamin A, C and E and selenium in IBD patients, this study is significant. This is a significantly large study on the prevalence of vitamin C deficiency in IBD patients but to say that a larger portion of IBD patients suffer this deficiency is over statement wherein only 21.6% of patients have this.

Response:

Thank you. We agree that noting that vitamin C deficiency exists in a large portion of IBD patients may have been an overstatement. We have revised this to better reflect the sentiment that vitamin C deficiency affects a significant, albeit minority, of IBD patients.

To address this, we have included the following in our discussion (page 11, paragraph 4): "Though many consider scurvy a historical disease of seafarers, the current study demonstrates that vitamin C deficiency affects a significant minority of IBD patients."

Comment:

One of the limitations of the study is that the study population of IBD is not specifically screened for vitamin C level but retrospectively included. And hence there is no data on other micro-nutrient deficiencies which may have bearing on IBD management.

Response:

Thank you. We agree that the retrospective nature of the study is a limitation and that ideally, these patients would have been prospectively screened for vitamin C deficiency.

We now acknowledge this limitation in our discussion (page 15, paragraph 1):

"Additionally, given the retrospective nature of our study, data are restricted to patients who had plasma vitamin C measurements available; these patients were not necessarily being screened for deficiency."

We also agree that it would be valuable to have data on other micro-nutrient deficiencies in these patients. For this reason, we now include the following:

Methods section (page 7, paragraph 2): "Vitamin B12 deficiency was defined as serum vitamin B12 level < 200 pg/mL or < 148 pmol/L[7]. Vitamin D deficiency or insufficiency was defined as serum vitamin D, 25-hydroxy level ≤ 20 ng/mL[23]."

Results section (page 9, paragraph 3): "Fifty-nine of 252 patients (23.4%) had iron deficiency, 1/264 (0.4%) had vitamin B12 deficiency, and 38/259 (14.7%) had vitamin D deficiency or insufficiency."

Results section (page 10, paragraph 2): "Iron deficiency (28.8% v. 20.2%, p=0.2), vitamin D deficiency/insufficiency (28.9% v. 21.3%, p=0.3), surgery (25.7% vs. 19.3%, p=0.2), and active smoking (50.0% v. 21.0%, p=0.1) were not associated with higher deficiency rates."

Additionally, we add this as a limitation in our discussion (page 15, paragraph 1):

"An additional limitation of this study was that measurements of all micronutrients were not performed."

Comment:

There is no data on marginal or significant deficiency. (Studies variously quote that 11 to 40 umol/L ,or 11 to 28 umol/L as marginal deficiency).

Response:

Thank you. We agree that incorporating data on patients with marginal deficiency or inadequate vitamin C levels (i.e. 11-28 µmol/L) will paint a more complete picture of vitamin C deficiency in IBD patients, highlighting those at risk of developing more severe deficiency.

To address this, we have included the following in our methods and results sections:

Methods section (page 8, paragraph 1): "Inadequate vitamin C level or marginal deficiency was defined as 11.4-28.0 µmol/L, consistent with prior studies[24,25]."

Results section (page 9-10, paragraph 4): "An additional 24.6% of IBD patients (74/301) had inadequate vitamin C levels (11.4-28.0 µmol/L)."

Comment:

Also the general risk factors for vitamin C deficiency are not analyzed in this study (Smoking is included in the table not in the text).

Response:

Thank you. We agree that we can be more explicit in our analysis of smoking as a risk factor for development of vitamin C deficiency.

To address this, we have added the following to our results section (page 9, paragraph 3) and incorporated additional analysis of smoking as a risk factor in Table 2.

"Six patients (2.0%) were active smokers and 42/291 (14.4%) had a BMI ≥ 30."

Furthermore, we have added the following later in our results section (page 10, paragraph 2).

"Iron deficiency (28.8% v. 20.2%, p=0.2), vitamin D deficiency/insufficiency (28.9% v. 21.3%, p=0.3), surgery (25.7% vs. 19.3%, p=0.2), and active smoking (50.0% v. 21.0%, p=0.1) were not associated with higher deficiency rates."

Comment:

There is no discussion on the pathophysiology of Vitamin C deficiency in UC, compared to CD which affects the absorptive areas of the micronutrients.

Response:

Thank you. We agree that further discussion of the pathophysiology of vitamin C deficiency in UC (as opposed to CD) is required.

To address this, we have added the following to our discussion section (page 12-13, paragraph 4):

"While previous studies report inadequate vitamin C intake in UC[31], to our knowledge, there are no prior studies describing proportions with vitamin C deficiency in UC. Vitamin C deficiency would be biologically plausible in CD as CD often affects the primary sites of vitamin C absorption in the small bowel. Interestingly, in UC patients [without small bowel disease], 16% had vitamin C deficiency. While dietary data was not available in this study, avoidance of vitamin C rich foods likely contributed to the development of vitamin C deficiency in patients with UC, as has been reported in previous studies[31]. Moreover, patients with UC often have elevated TNF-alpha, which has been shown to downregulate transporters involved in vitamin C uptake[13,14]."

Comment:

The described clinical features of vitamin C deficiency, except scurvy, are mostly non-specific and are multifactorial as quoted by the authors.

Response:

Thank you. We agree with this statement and now highlight this in our discussion (page 14, paragraph 2):

"This study also highlights the difficulty in making a diagnosis of vitamin C deficiency in patients with IBD. In our cohort, there was no difference in the presence of clinical features of scurvy in patients with vitamin C deficiency compared to those with normal vitamin C levels. Many sequelae of vitamin C deficiency are nonspecific and can mimic or coexist with active IBD, including fatigue, arthralgias, oral lesions, bleeding, poor wound healing, anemia, and iron deficiency[15]. Unfortunately, more specific findings in

scurvy, such as perifollicular hemorrhage and follicular hyperkeratosis, occur in only a small minority of vitamin C deficient patients, as our study reiterates."

Comment:

Though this study is claimed to be the first one to report, "vitamin C deficiency and endoscopic activity relationship" the number of patients who had endoscopy report available are only about one third of the study population.

Response:

Thank you. The current study presents data that includes 113 patients with endoscopic assessments and vitamin C levels. We agree that it is a minority of the study population; however, this represents a large study group nonetheless. In addition, in the rest of cohort, we provide objective assessment of intestinal inflammation using fecal calprotectin. We now clarify this in our discussion (page 12, paragraph 2):

"To our knowledge, this study uniquely examines the relationship between objectively quantified intestinal inflammation [using endoscopy, n=113, or fecal calprotectin, n=80] and vitamin C deficiency in a large cohort."

We also now address this further in the limitations portion of our discussion (page 14-15, paragraph 3):

"Though this study examined the relationship between endoscopic activity and vitamin C deficiency, analyses on this relationship were limited by the small number of patients with significant endoscopic inflammation (n=20)."

Comment:

In the discussion, the role of Vitamin C as an antioxidant may be added and also studies describing the role of antioxidant supplementation in IBD may be included in the references.

Response:

Thank you. We agree that vitamin C's role as an antioxidant is worth noting, especially given the inflammatory nature of IBD.

To address this, we have added the following to our discussion section (page 14, paragraph 2):

"Given the challenge of diagnosing scurvy in this population, providers should have a low threshold to test for vitamin C deficiency and counsel on adequate vitamin C intake. Unlike the relapsing and often refractory nature of IBD in many patients, vitamin C supplementation can lead to rapid resolution of symptoms, including some incorrectly ascribed to IBD. Even in IBD patients with unmeasured vitamin C levels, empiric supplementation is not unreasonable, given vitamin C's role as an antioxidant, preventing free radical damage and reducing extracellular oxidants[24]. However, future studies demonstrating that vitamin C supplementation can decrease inflammatory burden or improve clinical symptoms would be necessary prior to recommending empiric supplementation as standard of care for this population."

Reviewer #2:

Comment:

This study aims to report the prevalence of vitamin C deficiency in Inflammatory Bowel Disease (IBD). The authors discuss relevant results that could eventually be of interest in the IBD consultation. The manuscript is well written, cohesively and coherently organized, with a detailed description of the methods and results. The authors also present limitations to their study, adding to the strengthening of the manuscript. However, the authors should clarify some aspects regarding the Methods and Discussion sections. Major revisions: 1. Although the Methods section is written with detail, the authors do not mention if the manuscript was prepared according to the STROBE statement. If it was, this should be stated in the text, and a STROBE checklist should be provided as an Additional File.

Response:

Thank you. The study was prepared according to STROBE guidelines, and we acknowledge that incorporating this information strengthens our study.

To address this, we have provided a STROBE checklist as an additional file and added the following to our methods section (page 7, paragraph 1):

"The study was prepared according to a STROBE statement for cross-sectional studies, and is included in supplementary materials."

Comment:

2. In the "Patients Population" section, the authors state that "In patients with multiple plasma vitamin C levels, the lowest value and associated visit were utilized.". What is the reasoning behind this? Why did the authors not use a mean of all values that where below 11.4µmol/L? If there is a reasonable reason, it should be stated in the text.

Response:

Thank you. We selected the lowest value to identify patients who had ever experienced vitamin C deficiency. Single time points were used to accurately link to other variables (i.e. clinical disease severity, clinical symptoms of vitamin C deficiency, other laboratory values and inflammatory markers, endoscopic assessment, etc), whereas multiple timepoints would yield these invalid.

Comment:

3. As the authors stated, in IBD patients, vitamin C deficiency can be originated from insufficient consumption or malabsorption. As so, results on vitamin C deficiency should depend on the diet profile of each patient. However, the authors do not mention whether the blood was collected after a fasting period, neither do they mention if the composition of the last meal of each patient was assessed. How would one know if the results observed

are in fact, a consequence of IBD etiopathology, or a consequence of the patient's diet? If the blood was collected without any of these concerns, this should a limitation of this study and stated in the text.

Response:

Thank you. We agree that having dietary data would better elucidate factors associated with vitamin C deficiency. Unfortunately, given the retrospective nature of this study, dietary data was not available. Some lab manuals note that plasma vitamin C levels should be collected while fasting, although there is not robust data to suggest that fasting (or eating) affect same day plasma vitamin C levels.

To address this, we have acknowledged this limitation in our discussion section (page 15, paragraph 1):

"The retrospective nature of our study also limits our examination of whether inadequate consumption was associated with higher rates of deficiency, or whether fasting status at serum collection impacted vitamin C level, as dietary data was not available."

Comment:

4. Throughout the manuscript (Abstract-conclusion; Results; Discussion), it is mentioned that vitamin C deficiency is more prevalent in CD than UC. This is not true, since the numerically difference was not statistically significant. The text should be revised so that this numerically difference is not misleading to the readers. For example, in Discussion, line 3, "(…) with elevated rates in CD patients (24.4%)." is not accurate.

Response:

Thank you. We have corrected this in the abstract, results and discussion sections.

To address this, we have changed the verbiage in the following sections:

Abstract (page 4, paragraph 1): "Vitamin C deficiency was common in IBD~~, and particularly in CD~~."

Results (page 10, paragraph 1): "CD patients had numerically higher prevalence of vitamin C deficiency than those with UC, although this result did not reach statistical significance (24.4% vs. 16.0%, p=0.1, Figure 1)."

Discussion (page 11-12, paragraph 4): "In 301 patients, 21.6% of IBD patients had vitamin C deficiency, including 24.4% of CD patients and 16.0% of UC patients."

Discussion (page 12, paragraph 2): "Absolute rates of deficiency were non-significantly lower in UC, but numerical differences between UC patients with and without inflammation were similar to these differences in CD."

Comment:

5. It is mentioned in the Introduction that TNF-alpha downregulates transcription of transporters necessary for vitamin C uptake. The authors assessed the prevalence of vitamin C in patients under therapy with biologics (that includes drugs anti-TNF-alpha), but failed to discuss these results. How do the authors explain that biologic medication use is associated with increased rates of vitamin C deficiency? A subgroup of patients under therapy with anti-TNF-alpha biologics (infliximab, adalimumab, golimumab, and certolizumab pegol) should be created and analyzed.

Response:

Thank you. We agree that this is an important point to clarify. We briefly comment on the increased prevalence of vitamin C deficiency among those using biologics in our discussion, but we agree that this can be more fully explored, and that a separate analysis of those on TNF-alpha inhibitors is warranted.

To address, we have added the following analysis to our results section (page 10, paragraph 2):

"Among patients on current biologic therapy (n=133), there were higher proportions of vitamin C deficiency in those using TNF-alpha inhibitors (17/48) compared with those using non-TNF-alpha (16/85) biologics (35.4% v. 18.8%, p=0.03)."

Furthermore, we have added the following to our discussion section (page 13-14, paragraph 3):

"The association of current biologic medication use and vitamin C deficiency is less clear and may be due to this being a marker of a more severe disease course. In a subgroup analysis of patients using biologic therapy, patients on TNF-alpha inhibitors had higher rates of deficiency compared to those on non-TNF-alpha agents (i.e. vedolizumab, ustekinumab, etc.), which runs counter to our understanding of TNF-alpha in vitamin C deficiency. TNF-alpha is known to downregulate transcription of transporters required for vitamin C uptake[13,14], and thus, one might expect that patients using TNF-alpha inhibitors would have *lower*, not *higher* proportions of deficiency. This further supports the use of anti-TNF agents or biologics as a surrogate for disease severity. Future studies may be warranted to better investigate this mechanism."

Comment:

Minor revisions: 1. Throughout the manuscript, "umol/L" should be changed to "μmol/L".

Response:

Thank you. We agree.

To address this, we have switched "umol/L" to "μmol/L" throughout the manuscript.