

Response to Editor

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Column: Retrospective Study

Title: Predictors of vitamin D deficiency in Inflammatory Bowel Disease and health: A Mississippi perspective.

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Reviewer code: 02978155, 03662768 and 03662774

First decision: 2016-10-11 11:06

Scientific editor: Jing Yu

Dear Editor,

Thank you for taking the time to review in detail our above listed manuscript.

I have made the necessary changes to the format per your recommendations, added the mandatory statements, created the core tip and the audio core tip and added the comments section along with all its subheadings. In addition I have revised the abstract to meet the word count specifications.

I have updated the references accordingly and included all the authors, PMID and DOI (when available) in the bibliography section. Within the main text I have replaced () with superscript [] per your recommendations.

In addition I have made the changes per the reviewer's suggestions I have itemized the response starting on the next page. There were a considerable number of suggestions by one of the reviewers that required mere paraphrasing of sentences and using slightly different terminology. I have not highlighted those portions. We have however highlighted all other major changes/additions.

Reviewer # 3662768

General Comments

1 The term 'diagnosis' has been used throughout the manuscript. This is confusing as it leads the reader to believe that IBD patients are investigated at time of diagnosis. In addition, healthy control is not a diagnosis.

Response: We have reworded this to avoid any confusion

2 The authors should not refer to 'vitamin D levels' but rather 'vitamin D concentrations' (or 25(OH)D concentrations) This should be changed throughout the manuscript

Response: We have made this change throughout the MS

2 The P-value should be typed as: P = 0.001 and not P=0.001, with spaces. This should be changed throughout the manuscript

Response: We have made this change throughout the manuscript

Abstract

1 Background: suggest replacing 'can be a cause' with is thought to play a role in the pathogenesis of..

Response: Suggested changes made

2 Aims: replace 'the effect of diagnosis' with the association between diagnosis

Response: This phrase has been removed

3 Methods: Abbreviation for inflammatory bowel disease should be used here

Response: This has been corrected

4 Methods: The description of methods are not in line with the aims. The methods section describes an investigation into prevalence of vitamin D deficiency – this is not mentioned in the aims. Either way, the design of this study cannot determine prevalence as it is somewhat cross-sectional in nature.

Response: We have removed this phrase

5 Methods, second sentence: Suggest rephrasing to say: 'Logistic regression analysis was performed to determine the association between'

Response: This has been corrected

6 General: BMI must be written out and then abbreviated.

Response: This has been corrected

7 Results: Abbreviation for Crohn's disease (CD) should follow the word and then be used throughout the abstract.

Response: This has been corrected

8 General: The authors should not refer to 'vitamin D levels' but rather 'vitamin D concentrations'

Response: This has been corrected

9 General: The results should be typed as (51% vs 21% $p = 0.00001$) and not (51%vs21% $p=0.00001$), with spaces.

Response: This has been corrected

10 General: Rephrase: 'Those with Age >65 was more likely...' to say "Those with age > 65 years were more likely..."

Response: This has been corrected

Introduction

1 Paragraph one, second sentence: The etiology of IBD is thought to reflect innate and adaptive immune-mediated responses to luminal bacterial antigens leading to enhanced intestinal permeability and dysregulated intestinal immunity

Response: This has been re phrased as per reviewer's suggestion

2 Paragraph One, Sentence Four: Suggest rephrasing to state: 'numerous studies have demonstrated a link between low serum 25(OH)D concentrations and IBD, in both CD and UC patients'.

Response: This has been re phrased as per reviewer's suggestion

3 Paragraph One, Sentence Six; Suggest rephrasing to state: 'On the other hand, IBD patients may be at an increased risk for low vitamin D due to one or more of the following; insufficient dietary intake and Inadequate sun exposure'

Response: This has been re phrased as per reviewer's suggestion

4 Second paragraph, First sentence: no capitalization of word 'body mass index'.

Response: This has been corrected

5 Second Paragraph, Second sentence: replace are with 'is'

Response: This has been corrected

6 Final Paragraph: The aims must be clarified; for instance: 'We aim to determine the vitamin D status in an understudied cohort consisting of IBD and non-IBD patients and investigate the association between serum 25-hydroxyvitamin D (25[OH]D) concentrations and IBD diagnosis (CD and UC). In addition, this study aimed to investigate risk factors for vitamin D 'deficiency', namely race, gender, age, and BMI; as well as to compare vitamin D status with that of healthy controls.'

Response: This has been re phrased as per reviewer's suggestion

Materials and Methods

1 Comment: UMMC abbreviated before written out in paragraph

Response: This has been corrected

2 Comment: Use abbreviation for CD, UC and IBD consistently (paragraph one, sentence two)

Response: This has been corrected

3 Comment: 25(OH)D concentrations, not levels

Response: This has been corrected

4 Comment: More description must be given as to the type of facility. How many patients are seen at UMMC? Does it cater to the majority of IBD patients in Mississippi – or typically those who are more sick?

Additional details; public vs private health sector; demographics of patient population in general; satellite facility; referral based, etc.

Response: UMMC is a tertiary care center and the only academic medical institution in the state of Mississippi. Over half a million patient encounters are reported every year. It caters to both low and high acuity patients. Since this is a tertiary referral center, patients tend to be sicker. There is no reports for who sees all of the IBD patients in the state, but we see over 500 IBD patients annually. Less than 15 percent of the patients are uninsured and the majority have either public or private insurance. The distinction has not impacted the treatments we offer. All patient visits are in main campus in Jackson. We get patients through word of mouth and community referrals. We have added relevant portions of this paragraph in the methods section.

5 Comment: Was a piloted, standardized document used for data collection (i.e. the chart review)? If so, this must be described.

Response: A standard document was used to extract and record information however it was not piloted. We have added this to methods section

6 Comment: Authors state “Patients with an endoscopic diagnosis of IBD seen at the University of Mississippi Medical Center with available plasma 25(OHD) were included in the study” How can you diagnose CD, particularly UC endoscopically? In addition, data pertaining to disease duration is important and strongly recommend this be included as a variable. If possible, strongly recommend inclusion of medication use, surgical history smoking status and season of 25(OH)D status measurement into model.

Response: Itemized below

a) Regarding “Patients with an endoscopic diagnosis of IBD seen at the University of Mississippi Medical Center with available plasma 25(OHD) were included in the study”

This was a writing error. All diagnosis of IBD were made using clinical, endoscopic and histologic findings and the new language reflects that.

b) Given the retrospective nature of our study, there were some variables that could not be accounted for. In many patients we

struggled to find exact dates of symptom onset, history regarding smoking and alcohol use, objective assessment of symptoms including mayo clinical score or CDAI. Based on these issues we did not collect data regarding disease severity/need for surgery/complications/exact medication use etc. We understand that this is a major limitation of the study and have mentioned this under discussion. We do believe that the lack of this data does not undermine the validity of the presented data.

c) We Initially did not look in to seasonal variation, as we did not have an opportunity to interview these patients to get an accurate idea of their overall sun exposure (indoor workers vs outdoor workers vs inpatients). However since we did have the dates of vitamin D tests available, we performed analysis per reviewer's request to look at compare mean vitamin D concentrations during traditional winter and summer months. After excluding the outliers the mean concentrations were different however the p-value was 0.062 which is not significant. Admittedly, it is close enough to consider adding into the regression analysis, however this p-value is only using a partial dataset (based on seasonal analysis many patients are excluded) - therefore even if significant, it would not be enough to include in the regression on its own. We have now presented this under results.

7 Comment: More detail must be provided regarding the control population. For instance, why were only patients with malignancy excluded? What about other GI illness (other than IBD), or those with renal impairment, liver disease which can affect vitamin D concentrations. What was the reason for follow up in selected control patients? Controls patients are notably older than cases and predominantly female, suggesting these individuals were identified from a distinct sector of UMMC – this may introduce bias. Finally, was there any attempt to match controls (e.g., sex, age).

Response: As both databases were retrospectively designed, the controls are not matched. We did not report this project as a matched case-control for this reason. Patients were seen in the same suite at UMMC as the GI cohort; clearly, however, these patients are not necessarily followed by GI as would be any control. Older patients in a control cohort are expected considering the onset age of IBD in

general. As this database was already collected, the issues of renal/liver impairment, while valid, are unable to be accounted for. We could list this as a limitation is desired by the reviewers.

8 Comment: Did cases /controls have more than one plasma 25(OH)D measurement available? The season of measurement is also an important variable here and should be described in data (e.g., summer vs winter).

Response: Repeat vitamin D data is not available for controls. **211 IBD patients underwent vitamin D testing on 257 occasions.** 49 of 211 IBD patients had 2 available 25(OH)D measurements. A number we felt was too small to derive at meaningful conclusions. We have provided this information under results now in the first paragraph. Please see our response to comment 6c under materials and methods

9 Comment: Vitamin D Status Assessment subsection: Please note that there is no absolute consensus on cut-off values for vitamin D sufficiency and deficiency. This should be mentioned.

Response: We have now mentioned this in the main text

Statistics:

1 General: Include abbreviation for odds ratio (OR) – then abbreviate throughout manuscript.

Response: We have changed the manuscript accordingly

2 Paragraph One, Sentence Two: Suggest rephrasing to state: ‘...in this instance, our outcome of interest was ordinal and thus had three levels for vitamin D: deficient, insufficient and sufficient’.

Response: Changed per suggestion

3 Comment: The statistical paragraph is incomplete –has not included description of analysis performed in table 6.

Response: This was explained in the Regression Model section **Regression Model** We also investigated a cumulative logistic regression model that included age and race as covariates and the nine BMI categories. We did not include gender since it was not significantly associated with outcome or predictor. The model found

race remained significant ($p < 0.0001$), age was borderline significant ($p = 0.0715$), and there were significant differences between the nine BMI groups. To adjust for the multiple testing, we considered the proportions different only if $p < 0.002$, a conservative Bonferroni approach. Differences identified were the same as the stratified analysis in Table 6."

Results

4 First Paragraph, First Sentence: Please include patient numbers: '237 IBD patients (XX CD, XX UC) and 98 controls were identified'.

Response: Details have been added to the text

5 First paragraph: It is difficult to really say that "those with available 25(OH)D concentrations were more likely to have CD". Rather it may be more appropriate to state; those with CD were more likely to have a vitamin D concentration measured in our facility (for example).

Response: Changed per suggestion

6 Second Paragraph: Please provide numerical values with all percentages. Also, age and BMI should be represented as median and IQR here, and not as mean values.

Response: We have made the suggested changes in Table 2

7 Second Paragraph: What proportion of females were African American compared to the African American IBD females?

Response: 22/86 (25.6%) female Controls were AA compared to 60/125 (48%) IBD females.

8 Third Paragraph: suggest rephrasing to state: 'Demographics of the study population as a whole are shown in Table 3. Overall, there was a 2:1 female-to-male ratio and the majority of the subjects were Caucasian (61.2%) with a little over one-third African Americans (39.6%) and few of other races (1.9%). BMI was categorized into normal, overweight, and obese, with similar proportion of individuals in each category'

Response: This has been changed accordingly

9 Third Paragraph / Table 3: Demographics of the cohort needs to be represented in a separate table as cases (CD, UC) and controls –

and not combined. The data overall has been poorly represented. Vitamin D as an outcome should also be separated. With that being said, Table 3 is very confusing. I do not understand why cases and healthy controls have been grouped together as a single cohort for analysis. These groups should be evaluated separately – variables identified as significant are then used in logistic regression model.

Response: Separate demographics are provided in table 2. The intent behind table 3 was to yield an overall picture of the evaluated population. The populations are generally well balanced and thus are combinable in general. Therefore reporting Table 3 combined is reasonable. The primary measure of interest (vitamin D) is one of very few dissimilar characteristics between the IBD and control group. Thus the emphasis of our regression analysis was vitamin D.

10 Third Paragraph: the authors use the terms ‘Caucasian’ and ‘African American’ within the text, however in the table use the terms ‘White’ and ‘Black’. Use of one appropriate reference term should be used throughout for consistency.

Response: Changed per suggestion

11 Regression model subsection (first sentence): what is meant by a “cumulative logit logistic regression model”. Also, how did the authors create nine BMI categories?

Response: Logit is simply the terminology used in SAS and has been removed to avoid confusion. There are 3 BMI groups, but 9 "categories" when considering CD, UC, and control groups separately. We are amenable to suggestions related changing the word "categories".

12 Comment: The small sample size for the ‘other’ group does not permit meaningful analysis – statistical analysis should not be performed. Alternatively, perform an analysis as white vs non-white.

Response: We are amenable to removing the other group if the editors are in agreement with the reviewer, but included all data to be obvious in reporting with discrepancies. The white vs non-white comparison is unnecessary as Table 6 includes race as a variable in the multivariate analysis.

Table 1:

1 This table seems unnecessary and does not provide any meaningful data as it stands. Perhaps if authors can provide additional data on disease duration, number of follow up visits at UMMC, other GI/primary care providers, number of surgeries, medication use, smoking, season of 25(OH) measurement? There are too many outstanding variables to make this comparison. Also, age and BMI must be represented as median and IQR and not as mean and SD.

Response: We presented Table 1 to show the comparability of the two groups. Given the retrospective nature of the study and its inherent limitations, we felt that we should compare all available variables to detect any obvious confounding factors.

We are amenable to deleting Table 1 if editor and reviewer want us to.

We have presented Age and BMI as median and IQR now.

Please see our response under materials and methods 6b for the rest of this comment

Table 2:

1 As with table 1 this table requires additional data for any meaningful comparisons. Particularly given the considerably older age of the control group.

Response: As this database was already collected, many variables while valid, are unable to be accounted for. We could list this as a limitation if desired by the reviewers.

2 Comment: Left columns representing numerical and proportions should be followed by: no, (%). For example: Patients with CD, no(%) Patients receiving vitamin D supplementation, no., (%). This should be amended in all tables.

Response: The suggested changes have been made

Table 3:

1 Title: why is BMI described as 'a known risk factor'?

Response: This has been changed to modifiable risk factor

2 Within table: numerical values must be provided with percentages.

Response: The tables would be very busy if all the numeric values are written. The significantly different percentages have been bolded to provide the meaningful information without cluttering the tables. If editors agree with the reviewer, we can add these numbers to the table

1 Within table: Age should be followed by years (i.e. Age, years)

Response: Changes made per suggestion

2 Under Race: Do not understand why entire cohort was 'pooled' together for this analysis? (This was also performed for gender) This is not in line with the aims.

Response: Please refer to the response to comment # 9 under statistics

Table 4 / 5 :

1 Comment: Tables are incomplete. Analysis should be adjusted for gender and age.

Response: Of the four factors that appeared to be associated with plasma vitamin D concentrations, BMI is the only modifiable risk factor (which can be affected by race, gender and age). Therefore, we investigated the potential for confounding factors for the relationship of BMI with vitamin D by statistically testing the associations between BMI and non-modifiable risk factors: age, race and gender. (Table 4) Race, gender, and age can't be changed by outside influences.

2 Risk factors should be identified while adjusting for possible confounders; age, gender, ethnicity, BMI..

Response: Please see the previous question

3 The interaction of vitamin D supplementation with smoking status (if data available), age and season of vitamin D measurement should also be considered in the model.

Response: Please see previous response to similar question under material and methods. Comment 6b, 6c

Table 6:

1 I am not clear as to why the reference categories used in this table were chosen /modeled as they are.

Response: For the dichotomous variables in Table 6 (race, gender, diagnosis), the comparison is being made against a reference entry (for example, female versus male). In the case of diagnosis, the reference is control. In the case of gender, the reference is female. In the case of race, the reference is AA. The reference are labeled in Table 6.

2 Model should be adjusted for vitamin D supplementation, season of vit D status measurement, age and gender.

Response: Information on vitamin D supplementation was patchy therefore it was not included in the model. Please refer to previous response under Material and methods 6b, 6c for seasonal variation

References:

Some typographical mistakes

References: 9,10, 11, 26, 27

Response: We have reviewed these and believe that the reviewer is referring to the citation style. If Editors agree we can change these.

Comment: Introduction? In the following sentence: “faulty conversion of vitamin D to active metabolic forms; failure to conserve an adequate functional pool of vitamin D” you refer to Tajika M et al (ref 17). Ref 17 did not investigate the statement above, they only address the same point of view referring to: Compston JE, Creamer B. Plasma levels and intestinal absorption of 25-hydroxyvitamin D in patients with small bowel resection. Gut 1977;18:171–5. Please correct your reference.

Response: We have corrected this error

Comment: Material and methods ? Relevant parameters but it would have been interesting to include disease activity if possible since patients in remission is shown have another influence on vitamin D status than patients with active disease(1). ?

Response: Please see response to material and methods 6b. This is a limitation of the study.

Comment: I miss information of the control group. You state that “The control group consisted of patients without IBD or any active systemic disease that presented to UMMC and had plasma 25(OH) D levels obtained during routine follow up”. I will suggest that you include examples of diagnoses of these control patients, since they are still described as patients and not healthy subjects.

Response: As this database was already collected, the issues of renal/liver impairment, while valid, are unable to be accounted for. We could list this as a limitation is desired by the reviewers. Data was collected on HTN (69) AND DM (25). If editor thinks this is relevant, we can add it to results section

Comment: Results and discussion? The authors observed an interesting association between vitamin D deficiency and CD diagnosis in patients with BMI < 25. This group could comprise CD patients with server disease, increased surgery rates and weight loss. I recommend that the authors discuss this result in the discussion. ?

Response: Please refer to response under 6b, 6c

Comment: Disease activity is not included as a possible parameter for vitamin D deficiency. If possible, it would be very interesting to ad information of disease activity to your cohort as the grade of

inflammation may have higher influence on the vitamin D status than the diagnosis of IBD. If disease activity data is not available I will suggest that you address this limitation in the discussion. 1. Ham M, Longhi MS, Lahiff C, Cheifetz A, Robson S, Moss AC. Vitamin D levels in adults with Crohn's disease are responsive to disease activity and treatment. *Inflamm. Bowel Dis.* 2014;20(5):856-60.

Response: We will be addressing this in the limitations section. Please see 6b, 6c under Materials and methods

This is a well written manuscript on an interesting topic. Some sentences are not clear at all. Only as an exaple in the "core tip", the Authors wrote " The relationship between vitamin D is considered to be bidirectional." Probably this sentence could be clearer writing "The relationship between vitamin D AND INFLAMMATORY BOWEL DISEASE is considered to be bidirectional. ALso, in the title of the paper (Manuscript_20160904132509.docx)upload the Authors wrote "Predictors of vitamin D deficiency in inflammatory bowel disease health: A Mississippi Perspective": it's clearer the title version "Predictors of vitamin D deficiency in inflammatory bowel disease and health: A Mississippi Perspective" Generally, with some revision, it could be suitable for publishing

Response: We have made the appropriate corrections

I do hope that you will find the revised version to be satisfactory. In case of any concerns or deficiencies, I will be happy to make further changes.

I appreciate your time and effort in making this publication a possibility.

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