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OPEN PEER REVIEW

# Is There a Correlation Between Vitamin D Levels and Acute Diarrhea in Children?

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**About this report**

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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## 1 Original submission

5 comments · 17 Feb 2023 – 3 Mar 2023

### Referee 1 Reviewer

MINOR REVISION 17 Feb 2023

The manuscript has been well prepared.
The important point is the method of vitamin D measurement.
Did you measure vitamin D in a single reference lab?
How did you exclude the effects of confounding factors on the length of hospital stay?

### Referee 2 Reviewer

MAJOR REVISION 20 Feb 2023

Dear author
Thank you for your submission.
Overall, this is a valuable study with a good design. Please check the variables and their definitions and the inclusion and exclusion criteria to clarify the results and show that they are not confounded by different biases.
Abstract:
1-The total word count must be 250 words.
2-Please omit the sentence " Possibly, low vitamin D levels is a risk factor for acute non-inflammatory diarrhea." from the conclusion. This study cannot confirm this statement. We can explain the effect of vitamin D in this study, which may suggest a new hypothesis about vitamin D and diarrhea.
3-Please check the keywords against MESH.
Introduction:
1-Please give a brief explanation of vitamin D levels and non-bacterial diarrhea, with a reference, not just infectious diarrhea.
Method:
1- Were children with malnutrition or wasting and children with known vitamin D deficiency excluded from your cases? What about a history of supplementation with vitamin D?
2- What was the KIT number, and which country was the kit from? Please give information about the laboratory equipment used for vitamin D measurement. How did you reduce observer bias?
3- What was the method of blood sampling?
4- It is not suitable to give the table number from a textbook. Please give an appropriate reference and omit the table number and the name of the Nelson book.
5- Please give more explanation about the number of samples. How did you obtain the ratio of vitamin D deficiency in the control group (0.4) and the case group? Please give a reference for your rate assumption and the formula for the sample size calculation.
6- The use of ROC is confusing. Is it necessary to have an ROC curve in this study for vitamin D and diarrhea? Please check with your statistical adviser.
7- 6- Please give a briefer explanation of the statistical tests.
Result:
1- Please omit the titles in the different parts of the results section.
Discussion:
1- The discussion section should interpret the results based on a review of the literature, so please do not repeatedly explain the findings from the results section.
2- Based on your finding of lower vitamin D levels in non-inflammatory diarrhea, please give results from

more articles on this issue.
3- The ROC curve results and the sensitivity and specificity of vitamin D in the statistical findings are not mentioned in the discussion section.
4- Please give the advantages of your study.
Conclusion:
Please change this section according to the conclusion of the abstract.

Best regards

**Referee 3** Reviewer — MINOR REVISION — 22 Feb 2023

Dear colleague, I read your article. The article is accepted after two small changes are made.

1. In the title field, the correct wording is “vitamin d level,” not “vitamin d levels.”
2. What was the age range of the children studied: two to fourteen months or two to fourteen years?

**Referee 4** Reviewer — MAJOR REVISION — 3 Mar 2023

Dear author
1- The reason for calculating diagnostic indices (such as sensitivity and specificity) is not clear. This study is not designed according to the principles of diagnostic studies, and its purpose is to investigate the significance of the relationships. In addition, the vitamin D level is not a diagnostic indicator for diarrhea in children.
2- It is better to discuss the reasons for the difference in the results in the "Discussion" section. For example, what is the author's opinion about the fact that there was no difference in vitamin D levels between the groups, but the percentage of children with vitamin D deficiency in the acute non-inflammatory diarrhea group was higher than in the other groups? Also, the author's opinion should be expressed about the reasons for the increased length of hospitalization.

Regards

**Associate Editor** — MAJOR REVISION — 3 Mar 2023

Dear authors
Your manuscript has passed through the review stage and is ready for revision.

Editorial comments
To ensure the Editor and Reviewers can recommend that your revised manuscript be accepted, please pay careful attention to each of the comments posted underneath this email. This way we can avoid future rounds of clarifications and revisions, moving swiftly to a decision.

1. Please provide a point-by-point response to the Editor's and reviewer's comments.
2. Please highlight all the changes in your manuscript in yellow.
3. Use line numbering and page numbers in the next submission.
4. The English language is weak (to improve and maintain journal quality, the authors should use high-quality English).
6. There are some grammatical and spacing errors that need to be corrected throughout the manuscript.

First of all, the investigation of the serum vitamin D level and the duration of hospitalization as well as the degree of dehydration is not possible or useful based on the available data and should be omitted. The sensitivity and specificity analysis should be removed. I recommend that the authors compare all patients with diarrhea (both non-inflammatory and inflammatory diarrhea) in one group (cases) with control patients. The title and purpose of the study should be focused on the relationship between serum vitamin D levels and acute diarrhea in children without going into distracting and unnecessary details, including the duration of hospitalization and the degree of dehydration.
Other shortcomings:
In the revised version, remove the unfamiliar and unhelpful terms "Non-inflammatory and inflammatory diarrhoea" from the entire manuscript and use only the term "acute diarrhoea" instead.
Accordingly, a major revision must be made before the manuscript is considered for publication.

Other shortcomings
Spell out all abbreviations on first use.
Keywords should be written in alphabetical order.

2

# Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear reviewer one,

Thanks for the constructive and complimentary comments on our article,

1- According to your comment, "**The total word must be 250 words**" , we limited the abstract to 248 words.
2- According to your comment, " **please omit the sentence" Possibly, low vitamin D levels is a risk factor for acute non-inflammatory diarrhea." From the conclusion. This study cannot confirm this declaration. We can explain about the effect of vitamin D in this study which may shows new hypothesis on Vitamin D and Diarrhea**." , we omit this sentence in the abstract and conclusion.
3- According to your comment, " **Please check the keywords with MESH**", All the keywords were extracted from the MESH.
4- According to your comment, " **Please give a brief explanation about Vitamin D level and non-Bacterial Diarrhea with reference, not just infectious diarrhea**.", we added the following sentence in the introduction.

   Several studies from different countries have reported the relationship between low vitamin D levels and any kind of diarrhea in children.
5- According to your comment, " **Were children with malnutrition or wasted children and children with known vitamin D deficiency excluded from you cases? What about history of supplementation with Vitamin D.?**" , We also excluded malnourished children, children with a history of taking vitamin D supplements, and children with known vitamin D deficiency from the study, which were incorrectly missed in the manuscript. We apologize and thank you for pointing out this complimentary issue.
6- According to your comment, " **What was the KIT number and from which country? Please give information about the laboratory equipment for Vitamin D measurement? How did you reduce the observer bias?**" , we added this information in the method as stated below.

   Finally, the 25-hydroxyvitamin D (25(OH)D) level was measured in all samples by the Chemiluminescent immunoassays (CLIA) method and by the Architect Abbott i2000 immunoassay analyzer (USA). The person in charge of the test did not know which group the samples belonged to and only reported the results.

7- According to your comment, "**What was the method of blood sampling?**", we added this information in the method as stated below.

Then 5 milliliters of clotted blood were taken from each participant (the blood was collected from a superficial vein in the upper limb, generally the median cubital vein). The serum was separated by centrifugation at 3,000 rpm for 10 minutes and kept at − 20° Celsius until sufficient samples were collected for a batch test.

8- According to your comment, "**It is not suitable to give the table number in a textbook, plz give appropriate reference address and omit the table number and the name of nelson book.**", We removed the said part and cited the textbook.

9- According to your comment, "**Plz give more explanation about the number of samples. How did you obtain the ratio of vitamin D deficiency in the control group ( 0.4 )and case group. plz give reference on your rate assumption and the formula for sample size calculation .**", we edited the sample size section in the method as stated below:\

Based on the Mahyar et al. study(14), the frequency of vitamin D deficiency in the case group was 11.6%, and in the control group was 3.3%. We found that 74 cases for each group are needed to determine the relationship between vitamin D levels and acute diarrhea (power =85%, $\alpha$ =0.05).

10- According to your comment, "**Using ROC is confusing? Is it necessary to have ROC curve in this study for Vitamin D and Diarrhea? Plz check with your statistical adviser.**", we omit these parts in method and result.

11- According to your comment, "**Plz give a briefer explanation on statistical tests**", The statistical analyses part is summarized as follows.

In this study, the values of quantitative variables were shown as mean (standard deviation), and the values of qualitative variables were shown as frequency percentages. The Kolmogorov-Smirnov test was used to check the assumptions of normality of the vitamin D level. Kruskal-Wallis non-parametric analysis compared the average vitamin D level between three groups of children with acute inflammatory diarrhea, acute non-inflammatory diarrhea, and healthy children. Also, the prevalence of vitamin D deficiency among the three groups of children was compared using the chi-square test. Kruskal-Wallis non-parametric analysis was used to compare the average number of hospitalization days according to the severity of dehydration (mild, moderate, severe) in children with acute

diarrhea. Also, the relationship between the level of vitamin D (sufficient, insufficient, and deficiency) and the severity of dehydration (mild, moderate, severe) was investigated using a chi-square test. The data were analyzed using IBM SPSS Statistics version 24 and MedCalc software version 20.011. The significance level was considered at 0.05.

12- According to your comment, "**Plz omit the titles in different parts of result part**", we omit titles in the result.

13- According to your comment, "**Discussion part should give the interpretation of result part base on review of literature, so plz don't explain the result part findings repeatedly**", Thanks for your comment. But in most articles, a summary of the results is written in the first paragraph of the discussion, and then the points of the results are discussed in the following paragraphs. With your permission, the article will be complete if it is possible not to delete the first paragraph of the discussion.

14- According to your comment, "**Based on your finding on lower vitamin D level in non-inflammatory diarrhea, plz give more articles results in this issue.",** This comment was considered, and we cited an article in this field. But overall, studies in this area are mainly about acute diarrhea and inflammatory and bacterial diarrhea. Studies in non-inflammatory diarrhea are limited; this is one of our article novelties.

15- According to your comment, "**The results of ROC curve and Sen. And Specificity of Vitamin D in Statistical findings is not mentioned in the discussion part**.", as said befor we omit these parts in the method and result.

16- According to your comment, "**Plz give the advantages of your study**", we added the following paragraph to the discussion.

Multiple studies have been conducted on the relationship between vitamin D and acute diarrhea. Still, there are limited studies on the association between vitamin D and acute inflammatory or non-inflammatory diarrhea. The current study evaluated the relationship between vitamin D and acute diarrhea in the first step. In the next step, the relationship between vitamin D and acute inflammatory or non-inflammatory diarrhea was evaluated, which was one of the advantages of our study.

17- According to your comment, "**Plz change this part according to conclusion of abstract pa**rt", we edited conclusion same as abstract.

I hope the explanations and corrections made will be acceptable to you. Otherwise, I will wait for your comments to further improve this article. We are very eager that our article meets the conditions of publication in your journal and published in your prestigious journal.

Thanks again for your valuable and complementary comments.

With best regards

Dear reviewer two,

Thanks for the constructive and complimentary comments on our article,

1- According to your comment, " **In the title field : vitamin d level is correct not vitamin d levels**", we edited the title according to your comment.
2- According to your comment, "**What age group were the studied children, two to fourteen months or two to fourteen years?**", the children were 2 to 14 years. We edited the abstract according to this comment.

I hope the explanations and corrections made will be acceptable to you. Otherwise, I will wait for your comments to further improve this article. We are very eager that our article meets the conditions of publication in your journal and published in your prestigious journal.

Thanks again for your valuable and complementary comments.

With best regards

Dear reviewer three,

Thanks for the constructive and complimentary comments on our article,

1- According to your comment, "**The reason for calculating diagnostic indices (such as sensitivity and specificity) is not clear. This study is not designed based on the principles of diagnostic studies and its purpose is to investigate the significance of the relationships. In addition, vitamin D level is not a diagnostic indicator for diarrhea in children**.", we omitted these parts in the method and results.
2- According to your comment, " **It is better to discuss the reasons for the difference in the results in the "Discussion" section. For example, what is the author's opinion about the fact that there was no difference in vitamin D levels in the groups, but the percentage of children with vitamin D deficiency in the acute non-inflammatory diarrhea group was higher than in other groups? Also, the author's opinion should be expressed about the reasons for increasing the length of hospitalization**.", we added the following sentence in the discussion.

   Lower 25(OH)D levels may increase the severity of illness brought on by an infection.

   and

   Although in evaluation of vitamin D status, there was a higher frequency of vitamin D insufficiency in the non-inflammatory diarrhea group, there was no significant difference in serum vitamin D levels. It seems that this non-significance can be mainly due to the limited range of the vitamin D levels in patients.

I hope the explanations and corrections made will be acceptable to you. Otherwise, I will wait for your comments to further improve this article. We are very eager that our article meets the conditions of publication in your journal and published in your prestigious journal.

Thanks again for your valuable and complementary comments.

With best regards

Dear reviewer four,

Thanks for the constructive and complimentary comments on our article,

1- According to your comment, "**The important point is the method of vitamin d measurement. Did You measure this item in a single reference lab**.?", we added this information in the method as stated below.
   Finally, the 25-hydroxyvitamin D (25(OH)D) level was measured in all samples by the Chemiluminescent immunoassays (CLIA) method and by the Architect Abbott i2000 immunoassay analyzer (USA). The person in charge of the test did not know which group the samples belonged to and only reported the results.
2- According to your comment, "**How did you exclude the effects of confounding factors on the length of hospital stay?**", Thanks to your complimentary comment, unfortunately, this point was not considered in the study design and was one of the limitations of our study.

I hope the explanations and corrections made will be acceptable to you. Otherwise, I will wait for your comments to further improve this article. We are very eager that our article meets the conditions of publication in your journal and published in your prestigious journal.

Thanks again for your valuable and complementary comments.

With best regards

Dear Associate Editor,

Thanks for the constructive and complimentary comments on our article,

1- According to your comment, "**the investigation of the serum vitamin D level and the duration of hospitalization as well as the degree of dehydration is not possible or useful based on the available data and should be omitted**", The main aim of the current study was to investigate the relationship between vitamin D insufficiency and deficiency and acute diarrhea. Still, serum vitamin D level, the duration of hospitalization, and the degree of dehydration were added to the study as added values to assess all possible aspects. With your permission, we kept these parts as added value in the article. But if you still believe it is advisable to omit these items, we will omit them.
2- According to your comment, "**The sensitivity and specificity analysis should be removed.**", we omit these parts in method and result.
3- According to your comment, " **I recommend that the authors compare all patients with diarrhoea (both Non-inflammatory and inflammatory diarrhoea) in one group (case) with control patients. The title and purpose of the study should be focused on the relationship between serum vitamin D levels in children with acute diarrhoea without going into distracting and unnecessary details including the duration of hospitalization and the degree of dehydration.**", Thank you very much for your complementary comment. Given that there are many studies on the relationship between vitamin D and acute diarrhea, in our study, we divided acute diarrhea into two groups of inflammatory and non -inflammatory diarrhea (although this is also mentioned in Nelson's Textbook of pediatrics). In fact, this was the novility of our study that, unlike previous studies that merely evaluated the effect of vitamin D on acute diarrhea, we evaluated this relationship separately in this study. Of course, according to your valuable comment, we added a paragraph and a table in the result section inorder to investigate the relationship between vitamin D and acute diarrhea. The paraghraph is stated below:

   In the first step, the association between acute diarrhea and vitamin D levels was investigated. The results indicated that patients with acute diarrhea had lower vitamin D levels than healthy controls (p=0.04). In qualitative assessment, there were more patients

with deficient and insufficient vitamin D levels in the acute diarrhea group compared to the healty control, but this result was insignificant (p=0.146) (table 2).

4- According to your comment, “ **In the revised version, remove the unfamiliar and unuseful terms "Non-inflammatory and inflammatory diarrhoea" from the entire manuscript and use only the term "acute diarrhoea" instead**.”, Multiple studies have been conducted on the relationship between vitamin D and acute diarrhea. Still, there are limited studies on the association between vitamin D and acute inflammatory or non-inflammatory diarrhea. The current study evaluated the relationship between vitamin D and acute diarrhea in the first step. In the next step, the relationship between vitamin D and acute inflammatory or non-inflammatory diarrhea was evaluated, which was one of the advantages of our study.

   Given that there are many studies on the relationship between vitamin D and acute diarrhea, in our study, we divided acute diarrhea into two groups of inflammatory and non -inflammatory diarrhea (although this is also mentioned in Nelson's Textbook of pediatrics). In fact, this was the novility of our study that, unlike previous studies that merely evaluated the effect of vitamin D on acute diarrhea, we evaluated this relationship separately in this study. Of course, according to your valuable comment, we added a paragraph and a table in the result section inorder to investigate the relationship between vitamin D and acute diarrhea.

5- According to your comment, “**Spell out all abbreviations on the first occasion**.", we edited the manuscript based on this comment.

6- According to your comment, “**Keywords should be written in alphabetical order**.", we edited keywods and written them in alphabetical order.

I hope the explanations and corrections made will be acceptable to you. Otherwise, I will wait for your comments to further improve this article. We are very eager that our article meets the conditions of publication in your journal and published in your prestigious journal.

Thanks again for your valuable and complementary comments.

With best regards

## 2 Revision 1

1 comment · 13 Mar 2023

### Associate Editor

REJECT 13 Mar 2023

Thanks for submitting your revised manuscript. The author's responses to the reviewer's comments are unsatisfactory. I had hoped that the review would improve the manuscript to a level that could be accepted. Unfortunately, there are still significant deficiencies which cause the manuscript to fall short of that level. Except for some minor revisions, other main shortcomings remain unchanged.

3

# Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear reviewer one,

Thanks for the constructive and complimentary comments on our article,

1- According to your comment, "**The total word must be 250 words**" , we limited the abstract to less than 250 words.
2- According to your comment, " **please omit the sentence" Possibly, low vitamin D levels is a risk factor for acute non-inflammatory diarrhea." From the conclusion. This study cannot confirm this declaration. We can explain about the effect of vitamin D in this study which may shows new hypothesis on Vitamin D and Diarrhea**." , we omit this sentence in the abstract and conclusion.
3- According to your comment, " **Please check the keywords with MESH**", All the keywords were extracted from the MESH.
4- According to your comment, " **Please give a brief explanation about Vitamin D level and non-Bacterial Diarrhea with reference, not just infectious diarrhea**.", we added the following sentence in the introduction.

   Several studies from different countries have reported the relationship between low vitamin D levels and any kind of diarrhea in children.
5- According to your comment, " **Were children with malnutrition or wasted children and children with known vitamin D deficiency excluded from you cases? What about history of supplementation with Vitamin D.?**" , We also excluded malnourished children, children with a history of taking vitamin D supplements, and children with known vitamin D deficiency from the study, which were incorrectly missed in the manuscript. We apologize and thank you for pointing out this complimentary issue.
6- According to your comment, " **What was the KIT number and from which country? Please give information about the laboratory equipment for Vitamin D measurement? How did you reduce the observer bias?**" , we added this information in the method as stated below.

   Finally, the 25-hydroxyvitamin D (25(OH)D) level was measured in all samples by the Chemiluminescent immunoassays (CLIA) method and by the Architect Abbott i2000 immunoassay analyzer (USA). The person in charge of the test did not know which group the samples belonged to and only reported the results.

7- According to your comment, "**What was the method of blood sampling?**", we added this information in the method as stated below.

Then 5 milliliters of clotted blood were taken from each participant (the blood was collected from a superficial vein in the upper limb, generally the median cubital vein). The serum was separated by centrifugation at 3,000 rpm for 10 minutes and kept at − 20° Celsius until sufficient samples were collected for a batch test.

8- According to your comment, "**It is not suitable to give the table number in a textbook, plz give appropriate reference address and omit the table number and the name of nelson book.**", We removed the said part and cited the textbook.

9- According to your comment, "**Plz give more explanation about the number of samples. How did you obtain the ratio of vitamin D deficiency in the control group ( 0.4 )and case group. plz give reference on your rate assumption and the formula for sample size calculation .**", we edited the sample size section in the method as stated below:\

Based on the Mahyar et al. study(14), the frequency of vitamin D deficiency in the case group was 11.6%, and in the control group was 3.3%. We found that 74 cases for each group are needed to determine the relationship between vitamin D levels and acute diarrhea (power =85%, $\alpha$ =0.05).

10- According to your comment, "**Using ROC is confusing? Is it necessary to have ROC curve in this study for Vitamin D and Diarrhea? Plz check with your statistical adviser.**", we omit these parts in method and result.

11- According to your comment, "**Plz give a briefer explanation on statistical tests**", The statistical analyses part is summarized as follows.

In this study, the values of quantitative variables were shown as mean (standard deviation), and the values of qualitative variables were shown as frequency percentages. The Kolmogorov-Smirnov test was used to check the assumptions of normality of the vitamin D level. Kruskal-Wallis non-parametric analysis compared the average vitamin D level between three groups of children with acute inflammatory diarrhea, acute non-inflammatory diarrhea, and healthy children. Also, the prevalence of vitamin D deficiency among the two groups of children was compared using the chi-square test. The data were analyzed using IBM SPSS Statistics version 24 and MedCalc software version 20.011. The significance level was considered at 0.05.

12- According to your comment, "**Plz omit the titles in different parts of result part**", we omit titles in the result.

13- According to your comment, "**Discussion part should give the interpretation of result part base on review of literature, so plz don't explain the result part findings repeatedly**", Thanks for your comment. But in most articles, a summary of the results is written in the first paragraph of the discussion, and then the points of the results are discussed in the following paragraphs. With your permission, the article will be complete if it is possible not to delete the first paragraph of the discussion.

14- According to your comment, "**Based on your finding on lower vitamin D level in non-inflammatory diarrhea, plz give more articles results in this issue.",** This comment was considered, and we cited an article in this field. But overall, studies in this area are mainly about acute diarrhea and inflammatory and bacterial diarrhea. Studies in non-inflammatory diarrhea are limited; this is one of our article novelties.

15- According to your comment, "**The results of ROC curve and Sen. And Specificity of Vitamin D in Statistical findings is not mentioned in the discussion part**.", as said before we omit these parts in the method and result.

16- According to your comment, "**Plz change this part according to conclusion of abstract pa**rt", we edited conclusion same as abstract.

I hope the explanations and corrections made will be acceptable to you. Otherwise, I will wait for your comments to further improve this article. We are very eager that our article meets the conditions of publication in your journal and published in your prestigious journal.

Thanks again for your valuable and complementary comments.

With best regards,

Setila Dalili,

Dear reviewer two,

Thanks for the constructive and complimentary comments on our article,

1- According to your comment, " **In the title field : vitamin d level is correct not vitamin d levels**", we edited the title according to your comment.
2- According to your comment, "**What age group were the studied children, two to fourteen months or two to fourteen years?**", the children were 2 to 14 years. We edited the abstract according to this comment.

I hope the explanations and corrections made will be acceptable to you. Otherwise, I will wait for your comments to further improve this article. We are very eager that our article meets the conditions of publication in your journal and published in your prestigious journal.

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With best regards,

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Dear reviewer three,

Thanks for the constructive and complimentary comments on our article,

1- According to your comment, "**The reason for calculating diagnostic indices (such as sensitivity and specificity) is not clear. This study is not designed based on the principles of diagnostic studies and its purpose is to investigate the significance of the relationships. In addition, vitamin D level is not a diagnostic indicator for diarrhea in children**.", we omitted these parts in the method and results.
2- According to your comment, " **It is better to discuss the reasons for the difference in the results in the "Discussion" section. For example, what is the author's opinion about the fact that there was no difference in vitamin D levels in the groups, but the percentage of children with vitamin D deficiency in the acute non-inflammatory diarrhea group was higher than in other groups? Also, the author's opinion should be expressed about the reasons for increasing the length of hospitalization**.", we added the following sentence in the discussion.

   Lower 25(OH)D levels may increase the severity of illness brought on by an infection.

   and

   Although in evaluation of vitamin D status, there was a higher frequency of vitamin D insufficiency in the non-inflammatory diarrhea group, there was no significant difference in serum vitamin D levels. It seems that this non-significance can be mainly due to the limited range of the vitamin D levels in patients.

I hope the explanations and corrections made will be acceptable to you. Otherwise, I will wait for your comments to further improve this article. We are very eager that our article meets the conditions of publication in your journal and published in your prestigious journal.

Thanks again for your valuable and complementary comments.

With best regards,

Setila Dalili,

Dear reviewer four,

Thanks for the constructive and complimentary comments on our article,

1- According to your comment, "**The important point is the method of vitamin d measurement. Did You measure this item in a single reference lab**.?", we added this information in the method as stated below.
   Finally, the 25-hydroxyvitamin D (25(OH)D) level was measured in all samples by the Chemiluminescent immunoassays (CLIA) method and by the Architect Abbott i2000 immunoassay analyzer (USA). The person in charge of the test did not know which group the samples belonged to and only reported the results.
2- According to your comment, "**How did you exclude the effects of confounding factors on the length of hospital stay?**", Thanks to your complimentary comment, unfortunately, this point was not considered in the study design and was one of the limitations of our study.

I hope the explanations and corrections made will be acceptable to you. Otherwise, I will wait for your comments to further improve this article. We are very eager that our article meets the conditions of publication in your journal and published in your prestigious journal.

Thanks again for your valuable and complementary comments.

With best regards,

Setila Dalili,

Dear Associate Editor,

Thanks for the constructive and complimentary comments on our article,

1- According to your comment, "**Use line numbering and page number in the next submission**", we applied this comment.
2- According to your comment, "**The English language is weak (to improve and maintain journal quality, high-quality English language should be provided by authors)**", the manuscript was natively edited by an English language specialist.
3- According to your comment, "**There are some grammatical and spacing errors that need to be revised throughout the manuscript**", we applied this comment.
4- According to your comment, "**the investigation of the serum vitamin D level and the duration of hospitalization as well as the degree of dehydration is not possible or useful based on the available data and should be omitted**", we omit these parts in the method, result, and discussion parts.
5- According to your comment, "**The sensitivity and specificity analysis should be removed.**", we omit these parts in the method and result.
6- According to your comment, " **I recommend that the authors compare all patients with diarrhoea (both Non-inflammatory and inflammatory diarrhoea) in one group (case) with control patients. The title and purpose of the study should be focused on the relationship between serum vitamin D levels in children with acute diarrhoea without going into distracting and unnecessary details including the duration of hospitalization and the degree of dehydration.**", we applied this comment in the method, result, and discussion parts. The result of the manuscript was completely changed as stated below:

   A total of 222 children aged 2-14 were included in the current study, 148 children with acute diarrhea and 74 Healthy controls. The baseline characteristics of patients are shown in Table 1. The mean age of the participants was 5±2.7 years, and 114 of them (51.4%) were female. The mean age of healthy controls and acute diarrhea children were 5.53±3.18, 5.31±2.31, respectively.

   The results indicated that patients with acute diarrhea had lower vitamin D levels than healthy controls (p=0.04). In qualitative assessment, there were more patients with

deficient and insufficient vitamin D levels in the acute diarrhea group compared to the healthy control, but this result was insignificant (p=0.146) (table 2).

7- According to your comment, “ **In the revised version, remove the unfamiliar and unuseful terms "Non-inflammatory and inflammatory diarrhoea" from the entire manuscript and use only the term "acute diarrhoea" instead**.”, we applied this comment in the method, result, and discussion parts.

8- According to your comment, “**Spell out all abbreviations on the first occasion**.", we edited the manuscript based on this comment.

9- According to your comment, “**Keywords should be written in alphabetical order**.", we edited keywods and written them in alphabetical order.

10- According to your comment, “ **Thanks for submitting your revised manuscript. The author's responses are unsatisfactory to the reviewer’s comments. I had hoped that the review would improve the manuscript to a level that could be accepted. Unfortunately, there are still significant deficiencies which cause the manuscript to fall short of that level. Except for some minor revisions, other main shortcomings remain unchanged.**”, In the initial revision, the corrections requested by all the reviewers were corrected, and explanations were given. But regarding the Associate Editor's comments, we were asked to remove the terms "Non-inflammatory and inflammatory diarrhea" from the entire manuscript and omit the part of the investigation of the serum vitamin D level with the duration of hospitalization and the degree of dehydration.

Given that there are many studies on the relationship between vitamin D and acute diarrhea, in our study, we divided acute diarrhea into two groups of inflammatory and non -inflammatory diarrhea (although this is also mentioned in Nelson's Textbook of pediatrics). In fact, this was the novelty of our study that, unlike previous studies that merely evaluated the effect of vitamin D on acute diarrhea, we evaluated this relationship separately in this study.

On the other hand, investigating the relationship between the diarrheal patient's dehydration rate and the duration of their hospitalization with the vitamin D level was also one of the other innovations of this study. The presence of these items can increase the article's value to some extent.

According to Associate Editor's comments, we prepared this manuscript that does not include the duration of hospitalization, the degree of dehydration, and the separation of acute diarrhea into inflammatory and non-inflammatory diarrhea. The results of this manuscript have a total of two paragraphs, which only report the patients' demographic information and a quantitative and qualitative comparison between the two groups of diarrhea patients and the control group regarding vitamin D levels.
Based on our positive experience with our previous articles published in your journal, we greatly desire to publish this article in the Archives of Pediatric Infectious Diseases journal. We have prepared the new version of the manuscript, considering all the comments and corrections mentioned. If our explanation is still not convincing, please consider this manuscript version for review. Still, If the manuscript can have more complete parts, its value and efficiency will be higher.

I hope the explanations and corrections made will be acceptable to you. Otherwise, I will wait for your comments to further improve this article. We are very eager that our article meets the conditions of publication in your journal and published in your prestigious journal.
Thanks again for your valuable and complementary comments.
With best regards,
Setila Dalili,

## 3 Revision 2

1 comment · 12 Apr 2023

### Associate Editor

ACCEPT 12 Apr 2023

I read the revised manuscript.
I have no further comments to add. I thank the authors for their very detailed replies to my comments.