

## PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (<http://bmjopen.bmj.com/site/about/resources/checklist.pdf>) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Association Between Blood Circulating Vitamin D and Colorectal Cancer Risk in Asian Countries: A Systematic Review and Dose-Response Meta-analysis
<b>AUTHORS</b>	Zhang, Lin; Zou, Huachun; Zhao, Yang; Hu, Chunlei; Atanda, Adejare; Qin, Xuzhen; Jia, Peng; Jiang, Yu; Qi, Zhihong

### VERSION 1 - REVIEW

<b>REVIEWER</b>	Bruce W Hollis Medical University of South Carolina Charleston, SC 29464
<b>REVIEW RETURNED</b>	10-May-2019

<b>GENERAL COMMENTS</b>	<p>The authors have performed a very important meta-analysis with respect vitamin D and colon cancer in Asian countries. I believe the addition of a few references will make the study stronger.</p> <ol style="list-style-type: none"><li>1. Keum N et al, 2019; Annals of Oncology</li><li>2. Ng K et al, 2019; JAMA,</li><li>3. Marshall DT et al, 2011, JCEM 97:2315</li></ol>
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<b>REVIEWER</b>	Honghe Zhang Zhejiang University
<b>REVIEW RETURNED</b>	18-Jun-2019

<b>GENERAL COMMENTS</b>	<p>This manuscript performed a meta analysis to demonstrated that blood circulating Vitamin D level was associated with decreased risk of colorectal cancer in Asian countries. And the dose-response meta-analysis further showed that the power of the association among Asian populations was similar to that among western populations. However, some critical points need to be addressed.</p> <ol style="list-style-type: none"><li>1. In the primary analysis, it was demonstrated that blood circulating Vitamin D levels was inversely associated with risk of colorectal cancer including adenoma and carcinoma (OR=0.75, 95% CI: 0.58-0.97). However, stratified analysis by outcome showed neither colorectal adenoma (OR=0.67, 95% CI: 0.40-1.14) nor colorectal cancer (OR=0.83, 95% CI: 0.66-1.06) were associated with circulating Vitamin D concentrations. These results seems contradictory.</li><li>2. Of note, only one included study investigated the association between circulating Vitamin D levels and colorectal cancer risk in</li></ol>
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	<p>Western Asia. Because of the insufficient data of Western Asia, such subgroup was not rational.</p> <p>3. What was the aim for subgroup analysis by year of publication for the associations of circulating Vitamin D and the risk colorectal cancer and adenoma?</p> <p>4. The search strategy need to be improved. For example, some synonymous terms were missed, e.g. "tumor" or "carcinoma". In addition, "rectal cancer" should not be omitted which is also included in "colorectal cancer".</p> <p>5. The authors only included the studies reported in English. There might be studies published in other languages that are of good quality and worth including.</p>
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<b>REVIEWER</b>	Matteo Rota Department of Molecular and Translational Medicine, University of Brescia, Italy
<b>REVIEW RETURNED</b>	06-Aug-2019

<b>GENERAL COMMENTS</b>	<p>This reviewer should recognize that this is a well written manuscript with analyses conducted as per state of the art. Of course, the topic is not novel and there are several published systematic reviews and meta-analyses investigating the association between vitamin D levels and colorectal cancer risk. The partial novelty of this meta-analysis is the focus on the study of the association among Asian populations only. I have no concerns with reference to the statistical methods and analyses used, but only a few minor suggestions to improve the manuscript.</p> <p>Minor points:</p> <p>Page 3 line 7-8: typo, "dose" instead of "dose"; "vitamin D levels" missing "s".</p> <p>Page 9 line 43-47. The highest versus lowest category meta-analysis is not, and could not be, performed to test the fit of the non-linear model. These two analyses are independent. I suggest to avoid to state "To test and verify the non-linear model".</p> <p>Page 14 lines 34-38: please rewrite the sentence "with the a meta-analysis from ...".</p> <p>Table 3. Please clarify in the table footnote if the I2 and P-value are related to subgroup differences.</p> <p>Figure 2, Figure S1A-S1F. Typo: country for Yurekli et al. study "Turkey" not "Turky". Please also better identify the "range" column within Figure 2 and S1F by specifying also the unit measure: vitamin d range (ng/ml).</p> <p>Figure 3. The y axis does not appear to be on a log-scale as declared. Please check. Moreover, I would specify in the figure that the best model is the linear one by reporting the P-value for non-linearity. This is because generally only the most parsimonious model should be reported, but in this case I retain useful to see both.</p>
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### VERSION 1 – AUTHOR RESPONSE

Reviewer #1:

The authors have performed a very important meta-analysis with respect vitamin D and colon cancer in Asian countries. I believe the addition of a few references will make the study stronger.

1. Keum N et al, 2019; Annals of Oncology
2. Ng K et al, 2019; JAMA,
3. Marshall DT et al, 2011, JCEM 97:2315

Response: Thanks for reviewer's suggestion to include the 3 references to this manuscript. After carefully reading the references, we agree with the reviewer that the 3 references add the scientific value for the interpretation of the study and future research. Therefore, in the revised version, the suggested references were added in the discussion section on page 15 and highlighted in yellow.

Reviewer #2:

This manuscript performed a meta analysis to demonstrated that blood circulating Vitamin D level was associated with decreased risk of colorectal cancer in Asian countries. And the dose-response meta-analysis further showed that the power of the association among Asian populations was similar to that among western populations. However, some critical points need to be addressed.

1. In the primary analysis, it was demonstrated that blood circulating Vitamin D levels was inversely associated with risk of colorectal cancer including adenoma and carcinoma (OR=0.75, 95% CI: 0.58-0.97). However, stratified analysis by outcome showed neither colorectal adenoma (OR=0.67, 95% CI: 0.40-1.14) nor colorectal cancer (OR=0.83, 95% CI: 0.66-1.06) were associated with circulating Vitamin D concentrations. These results seem contradictory.

Response: Thanks for the reviewer to point out this very important result needed to be interpreted well. With the overall analysis for both adenoma and carcinoma, the association was statistically significant; yet, in the stratified analysis by colorectal adenoma or colorectal cancer, the association was not statistically significant; The results suggested that associations were in the same direction (inverse relationship), the difference from the results was the effect size and corresponding 95% CI. The possible reason for this seemingly contradictory result, was coming from the sample size of the stratification of colorectal cancer (1,268 cases) and adenoma (1,648 cases), therefore the 95% CIs of the effect size is wider than the overall analysis (2,916 cases).

2. Of note, only one included study investigated the association between circulating Vitamin D levels and colorectal cancer risk in Western Asia. Because of the insufficient data of Western Asia, such subgroup was not rational.

Response: Thanks for the reviewer's suggestion to remove the subgroup analysis. With careful consideration, we suggest keeping the subregion analysis of regions in Asia, the rationales are as following: Although, the number of studies from Easter Asia (7 studies) and Western Asia (1 studies) are imbalance, in our study, we want to reveal the important difference of studies in Asian subregion, because the culture and lifestyle of the different regions in Asia are diverse, therefore type of food consumption, dietary habits and nutrients, as well as exposure to sunlight are strongly associated with the blood Vitamin D levels, and subsequently alter the association of blood Vitamin D levels and colorectal cancer. This information may also important for the subregion of Asia to implement region-specific colorectal cancer prevention program and strategy in general.

3. What was the aim for subgroup analysis by year of publication for the associations of circulating Vitamin D and the risk colorectal cancer and adenoma?

Response: Thanks for reviewer's suggestion for subgroup analysis of year of publication. The reason we conducted the subgroup analysis was because we want to avoid the bias from the year of population (we supposed that in some year the publication was more than other years). We agree the reviewer's suggestion, that the year of publication is not strongly relevant to the aim of this study, so we removed the subgroup analysis of year of publication in Table 3 and responding sentence in the statistical analysis and results (subgroup analysis) on page 10 and page 12, as well as the supplementary Figure S1A.

4. The search strategy need to be improved. For example, some synonymous terms were missed, e.g. "tumor" or "carcinoma". In addition, "rectal cancer" should not be omitted which is also included in "colorectal cancer".

Response: Thanks for reviewer's suggestion to improve the search strategy. During the study design phase, before we performed the search strategy, we consulted with the search strategy expert from the library of the Peking Union Medical College for not missing important publications, therefore our current strategy was based on the result of discussion with the academic librarian. With reviewer's suggestion, we consulted with another academic librarian in the same institute for the search strategy, who suggested that we could check the PubMed MeSH term category.

On the PubMed MeSH term category, both "tumor" or "carcinoma" equals to MeSH term "neoplasms" and "colorectal neoplasms" includes rectal neoplasms (rectal cancer is in this category). You can see

Tree Number(s): C04  
MeSH Unique ID: D009369  
Entry Terms:

- Neoplasia
- Neoplasias
- Neoplasm
- Tumors
- Tumor
- Cancer
- Cancers
- Malignancy
- Malignancies
- Malignant Neoplasms
- Malignant Neoplasm
- Neoplasm, Malignant
- Neoplasms, Malignant
- Benign Neoplasms
- Neoplasms, Benign
- Benign Neoplasm
- Neoplasm, Benign

the following snapshots from MeSH term categories of PubMed.

[All MeSH Categories](#)  
[Diseases Category](#)  
[Neoplasms](#)  
[Neoplasms by Histologic Type](#)  
[Neoplasms, Glandular and Epithelial](#)  
**Carcinoma**

[All MeSH Categories](#)

[Diseases Category](#)

[Digestive System Diseases](#)

[Gastrointestinal Diseases](#)

[Gastrointestinal Neoplasms](#)

[Intestinal Neoplasms](#)

**Colorectal Neoplasms**

[Adenomatous Polyposis Coli](#)

[Gardner Syndrome](#)

[Colonic Neoplasms](#)

[Sigmoid Neoplasms](#)

[Colorectal Neoplasms, Hereditary Nonpolyposis](#)

[Rectal Neoplasms](#)

[Anus Neoplasms](#) +

5. The authors only included the studies reported in English. There might be studies published in other languages that are of good quality and worth including.

Response:

Thank you for the reviewer's suggestion to include other languages in the search strategy for not missing any quality studies. We considered the searched publication language restricted to English during our study design phase for eligibility criteria (you can see this in Eligibility criteria on page 7 highlighted in yellow), because we did not have the translator resource for all languages and include the studies in each language. Therefore, we decided that only publication in English to be included in our systematic review and meta-analysis. Of course, excluding publication in other Asian languages is a limitation

Reviewer # 3

This reviewer should recognize that this is a well written manuscript with analyses conducted as per state of the art. Of course, the topic is not novel and there are several published systematic reviews and meta-analyses investigating the association between vitamin D levels and colorectal cancer risk. The partial novelty of this meta-analysis is the focus on the study of the association among Asian populations only. I have no concerns with reference to the statistical methods and analyses used, but only a few minor suggestions to improve the manuscript.

Minor points:

1. Page 3 line 7-8: typo, "dose" instead of "dose"; "vitamin D levels" missing "s".

Response:

Thanks for the reviewer to point out the typo in our manuscript. These typos were solved on Page3 highlighted in yellow.

2. Page 9 line 43-47. The highest versus lowest category meta-analysis is not, and could not be, performed to test the fit of the non-linear model. These two analyses are independent. I suggest to avoid to state “To test and verify the non-linear model”.

Response:

Thanks for the reviewer’s suggestion. We agree that the statement is not relevant and should not be stated this way. Therefore, “To test and verify the non-linear model” was avoided by removal and the sentences were restructured and highlighted in yellow on page 9.

3. Page 14 lines 34-38: please rewrite the sentence “with the a meta-analysis from ...”.

Response:

Thanks for the reviewer’s careful reading and pointing out this sentence for . We rewrote the sentence as following:

“...consistent with the result of a meta-analysis from Ekmekcioglu C et al <sup>13</sup> , which revealed a pooled RR of 0.62 (0.56–0.70) for colorectal cancer when comparing individuals with the highest category of 25(OH)D with those in the lowest.”

We also highlighted rewritten sentence in yellow on page 14.

4. Table 3. Please clarify in the table footnote if the  $I^2$  and P-value are related to subgroup differences.

Response:

Thanks for reviewer’s suggestion to add a statement of the interpretation of  $I^2$  and P-value. The clarification is stated in the footnote of Table 3 highlighted in yellow on page 24.

5. Figure 2, Figure S1A-S1F. Typo: country for Yurekli et al. study “Turkey” not “Turky”. Please also better identify the “range” column within Figure 2 and S1F by specifying also the unit measure: vitamin d range (ng/ml).

Response:

Thanks for reviewer’s suggestion for the typo and missing unit. We solved typo of “Turkey” in each figure (including Figure 2 and each figure in the supplementary), and we added the range unit by remaking the figure.

6. Figure 3. The y axis does not appear to be on a log-scale as declared. Please check. Moreover, I would specify in the figure that the best model is the linear one by reporting the P-value for non-linearity. This is because generally only the most parsimonious model should be reported, but in this case I retain useful to see both.

Response:

Thanks for reviewer's comments. We rechecked the vertical axis (y axis) which is on a log-scale, you may see from the bar between 0.4-0.6 is not as long as 0.6-0.8, as well as the following bars.

Thanks for pointing out that the best model is the linear one, which we agreed with. Therefore, we added the  $P_{\text{non-linearity}}=0.11$  in the statement of Figure 3 and highlighted in yellow, as well as we added a statement in the result (Meta-analysis and dose-response analysis) section on page 12: "Therefore, the linear model is the more fitted mode in this dose-response relationships, and highlighted in yellow."

#### VERSION 2 – REVIEW

<b>REVIEWER</b>	Honghe Zhang Zhejiang University School of Medicine, China
<b>REVIEW RETURNED</b>	23-Sep-2019

<b>GENERAL COMMENTS</b>	Thanks for the authors to response every point of my concern. But for the overall analysis for both adenoma and carcinoma, the association was statistically significant; yet, in the stratified analysis by either colorectal adenoma or colorectal cancer, the association was not statistically significant. Although the author believed the possible reason for this contradictory result was coming from the sample size of the stratification of colorectal cancer (1,268 cases) and adenoma (1,648 cases), obviously, such explanation was not convincing enough. I suggest that more explanations to address the point in this study before publication.
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<b>REVIEWER</b>	MATTEO ROTA Department of Molecular and Translational Medicine, University of Brescia, Italy
<b>REVIEW RETURNED</b>	06-Sep-2019

<b>GENERAL COMMENTS</b>	The authors addressed my comments. The manuscript is suitable for publication in BMJ Open.
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## VERSION 2 – AUTHOR RESPONSE

Reviewer #2:

Thanks for the authors to response every point of my concern. But for the overall analysis for both adenoma and carcinoma, the association was statistically significant; yet, in the stratified analysis by either colorectal adenoma or colorectal cancer, the association was not statistically significant. Although the author believed the possible reason for this contradictory result was coming from the sample size of the stratification of colorectal cancer (1,268 cases) and adenoma (1,648 case), obviously, such explanation was not convincing enough. I suggest that more explanations to address the point in this study before publication.

Response:

We agree with the reviewer that sample size alone shouldn't be the cause of this difference in significance, we list the possible reasons to explain this contradictory result.

1. Unexplained/unidentified confounders in the original studies mined are likely partially responsible for these differences. As an example, dietary sources of Vit D - consumption of fish/fibres containing 25(OH)D, exposure to the sun, folate/calcium intake, Vit D supplement use etc - Only one of the 8 studies in our meta-analysis adjusted for these confounders (Otani et al. 2007). We mentioned this reason in the discussion for this difference in significance level.
2. Another hypothesis for the explanation can be the presence of negative confounders (OR's are closer to 1) in original colorectal cancer studies, and positive confounders (OR's further from 1) in original colorectal adenoma studies as well as some effect modifications in both subgroups. We used random effects model in our analysis to reduce this effect.
3. Sample size of the stratification of colorectal cancer (1,268 cases) and adenoma (1,648 cases) is smaller than in the overall analysis (2,916 cases,). The results suggested that associations were all in the same direction (OR<1 indicating an inverse relationship), however, the seemingly contradictory result comes from the effect size and corresponding 95% CI. The larger the sample size, the more accurate information we can have and so our uncertainty reduces-narrows down our 95% CI in the overall analysis (OR=0.75, 95% CI: 0.58-0.97).
4. We further investigated the subgroup analysis and found that two studies (Budhathoki et al. and Takahashi et al.) should be important contributors for the inconsistency, because their weight is large in the subgroup meta-analysis. However, in the overall analysis, the weight of two studies is smaller. Therefore we suppose that the influence of the two studies is reduced, and the overall result is easier to be significant.

Reviewer #3:

The authors addressed my comments. The manuscript is suitable for publication in BMJ Open.

Response: Thanks for reviewer's comments and support. We are delighted that with the efforts of both reviewers and authors, this manuscript is suitable for publishing on the BMJ Open.