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Title: Circulating levels of vitamin D and colorectal adenoma: A case-control study and a meta-analysis

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We would like to thank you for inviting us to resubmit a revised copy of the manuscript entitled “Circulating levels of vitamin D and colorectal adenoma: A case-control study and a meta-analysis“ for consideration for publication in the World Journal of Gastroenterology. We also appreciate the time and insightful comments provided by you and by each reviewer and have incorporated the suggested changes into the manuscript.

We have carefully revised the original manuscript according to the suggestions and comments by editors and by reviewers. Accordingly, we have uploaded a copy of the original manuscript marked with the changes by using the yellow highlighter tool in Word. Appended to this letter is our point-by-point response to the comments raised by editors and by reviewers.

We believe that the revised manuscript has been greatly improved and hope that this is accepted for publication in the World Journal of Gastroenterology.

Sincerely,

Jung Eun Lee, Sc.D.

Reviewer 1

This is a well written manuscript in in writing and structure. The authors performed a case-control study among Korean adults determining the association between colorectal adenoma and 25(OH)D levels, moreover they systematically summarized the studies that have been made previously. However, according to the retrospective nature, the conclusion of this case-control study and the meta-analysis should be carefully explained. Furthermore, some changes are suggested below: Introduction: The reasons why serum 25-hydroxyvitamin D was measured for calculation but not 1,25-dihydroxyvitamin D should better be explained briefly in the case-control study.

We appreciate the reviewer's points. We have added the following statement to explain the measurement of 25-hydroxyvitamin D

Page 6 "However, 1,25(OH)2D may not be a good indicator of vitamin D because it is tightly regulated largely by various factors {Institute of Medicine, 1997 #205}"

Actually, the inverse association between circulating vitamin D levels and colorectal adenoma has been reported in the previous meta-analyses including one meta-analysis published in 2011 authored by the corresponding author of this manuscript. [Lee J E. Circulating levels of vitamin D, vitamin D receptor polymorphisms, and colorectal adenoma: a meta-analysis[J]. Nutrition research and practice, 2011, 5(5): 464-470.] The difference should better be stated if possible. What new questions does this manuscript try to answer?

Unlike the previous meta-analysis, we were able to examine the association among East

Asian population because of additional East Asian studies. We have address this point in the introduction as follow;

Page 7. "Although a previous meta-analysis reported a potential inverse association between circulating vitamin D levels and colorectal adenoma{Lee, 2011 #203}, a limited number of Asian studies did not allow to examine whether this association observed was applied to Asian populations."

Materials and Methods: In the case-control study, the sample size is not strong in such kind of study. The authors conducted the case-control study from August 2011 to September 2012, however, blood samples were provided between January and February 2013, long time after the colonoscopy. How could the bias been controlled as the concentration of blood (25OHD) levels MAY change long time after the colonoscopy.

We agree with the reviewer's points. Although we were not able to examine the changes in vitamin D levels, we think that their vitamin D levels have not been changed dramatically. Several reasons can be suggested 1) clinicians did not recommend any lifestyle changes related to vitamin D levels. Thus change in vitamin D levels may not be different by case status; 2) After colonoscopy in autumn, they came to the clinic in winter for blood draw. A few months in autumn and winter may not dramatically influence their vitamin D levels; and 3) a single measure of vitamin D metabolites has been known to be a useful marker to reflect long-term vitamin D status (Hofmann JN et al. CEBP 2010; Platz EA et al. CCC 2004; Kotsopoulos J et al. CEBP 2010).

In the case-control study of Korean adults, the authors found an inverse association between circulating serum 25(OH)D levels and colorectal adenoma in women but not in men. Similar results in women but not in men were also found in literature. In table 3, Aigner' study (2014, Austria) was marked as "W". Actually, there were males in Aigner' study. Will this affect the stratification by sex?

Although Aigner's study included both men and women, they reported ORs for vitamin D levels for only women because the association was only significant for women (Aigner E et al. Aliment Pharmacol Ther 2014). Although we contacted the author and received information that unit per increment was 1 ng/mL in the Table 4 (it was not noted in the article), we did not expect them to newly analyze male population for ORs of vitamin D levels. Therefore, we included only women as presented.

Results: The results were stated clearly. Discussion: The discussion should better be improved. The difference compared with previous studied could better be explained. The retrospective nature of the study is a limitation, too. Paragraph 4: "In an in vitro study, vitamin D treatment in Wistar rats reduced the apoptosis in colon tumors." Study in wistar rats should be an in vivo study. Apoptosis reduction is beneficial for tumor formation.

We appreciate the reviewer's comments. We have revised the discussion part as follows and corrected the error.

Page 13 "Although we found an inverse association between circulating vitamin D levels and colorectal adenoma in a previous meta-analysis [16], a limited number of Asian studies did not allow us to explore whether high vitamin D was associated with a lower

prevalence of colorectal adenoma in Asian populations along with Western populations. Because more studies have been published and we added our study, we examined the potential benefit of vitamin D against early stage of colorectal cancer in Asian populations. It needs further prospective studies."

Page 14 "Also, because our study participants provide blood samples after colonoscopy, vitamin D levels could have been changed if participants altered their lifestyle such as outdoor activities and dairy food intake. Especially, if vitamin D levels in participants with adenoma increased, the association would have been attenuated toward no association. However, an inverse association in a meta-analysis may suggest that an inverse association we observed in women may not be a seriously biased result. We cannot rule out the possibility that no association in men could be partly due to the retrospective nature in our study."

Reviewer 2

This is a good paper. The authors need though to add to their discussion one extra limitation to the study. Being retrospective, they (and the same holds I assume for most or all of the other studies used in the meta-analysis) are measuring blood (25OHD) levels many months after the colonoscopies are performed. The assumption is that these blood levels are approximately the same as what these patients had during the years in which their adenomas were developing. Perhaps. But consider the patients are older, may have altered lifestyle as a result of a positive adenoma test, etc. More optimal would have been a study in which blood was drawn before the test, and even that doesn't necessarily give a long-term view of blood

levels during the period in which these adenomas develop. This of course does not invalidate the study, but it does require the limitation to be duly noted in the discussion.

We appreciate the reviewer's comments. We have revised the discussion part as follows;

Page 14 "Also, because our study participants provide blood samples after colonoscopy, vitamin D levels could have been changed if participants altered their lifestyle such as outdoor activities and dairy food intake. Especially, if vitamin D levels in participants with adenoma increased, the association would have been attenuated toward no association. However, an inverse association in a meta-analysis may suggest that an inverse association we observed in women may not be a seriously biased result. We cannot rule out the possibility that no association in men could be partly due to the retrospective nature in our study."