PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Association between vitamin D and uterine fibroids: a study protocol of an open-label, randomised controlled trial
AUTHORS	Bo, Sheng; Song, Yizuo; Liu, Yi; Jiang, Chenchen; Zhu, Xueqiong

VERSION 1 – REVIEW

REVIEWER	Z. Asli Oskovi-Kaplan Ministry of Health Ankara City Hospital
REVIEW RETURNED	17-Apr-2020

GENERAL COMMENTS	The manuscript is subjected on a popular topic, Vitamin D, which is also proved to have influence on uterine leiomyomas. Previous studies have demonstrated that vitamin D wa ssignificantly lower in uterine leiomyomas. Yet, a well-designed prospective study on the preventive effect of vitamin D for uterine leiomyomas has not been published. This study design aims to evaluate this preventive effcect of vitamin D. The English language usage is appropriate, only few spelling errors needs to be corrected. My majör concern is that, vitamin D has an important role one many metabolic pathwats and some group of patiens will not be treated for a 2 years period. The ethical issue shall be re-considered. As far, i haven't noticed the patients' body mass index in the inclusion or exclusion criteria. Vitamin D levels also may be influenced by the BMI, so i would rather this criteria should be considered.

REVIEWER	Ferrero, Hortensia IVI Foundation, Valencia (Spain)
REVIEW RETURNED	29-Apr-2020

GENERAL COMMENTS	The objective of presenting a manuscript to a scientific journal is to show new information about a scientific topic, as well as to discuss results obtained in order to contribute new knowledge to the scientific community. However, this manuscript does not present any results about the proposed objective. This manuscript is in the process of recruiting patients and no data has yet been collected. Since the purpose of scientific journals, such as BMJ Open Journal, is to publish scientific results to contribute to scientific knowledge, it make no sense to accept a manuscript in which the objective and experimental desing of the study is only described, without providing any results and discussion.

REVIEWER	Michal Ciebiera Center of Postgraduate Medical Education, Warsaw, Poland

REVIEW RETURNED	06-May-2020

GENERAL COMMENTS	Dear Authors I have read your article with great interest. I am happy that the idea of vitamin D use in uterine fibroids is studied all over the world. I am happy with this trial as we need randomized controlled trials to found if we can treat fibroids with this compound. I have some comments I wuld like to discuss: Abstract should be rewritten after the full review adding some new things from points discussed later (there is also no info about the method of measurement in abstract). Another limitation (which is a strenght some way) One population - only Chinese women. Page 4 Line 10 - not exactly, there are also groups of women with less incidence like 25% etc. Line 15 - as you did not add the per year ingo this is misleading with the sentence above. Please rewrite this part about the epidemiological data. Line 25 UAE is not a method of choice and cannot be treated the same place like myomectomy and hysterectomy. You can mention it as a second line method (e.g. like in your reference Vilos et al. 2015 Line 29 Please add examples of existing GnRH analogs, you can stay uptodate with the new oral ones. Please provide the group for mifepristone, however I am not sure if this a compound frequently used to treat fibroids. I would rather placed SPRMs here, as they were used for some years with good overall results. Page 5 Line 10 I would add the recent data by Corachan et al. 2019 and 2020 about vitamin D and fibroids. Line 12-15 I am not sure if you should add this info. This data is unpublished and there is plenty of data about this that could be directly read by a interested reader. Line 25 Please check the very recent data by Arjeh et al. 2020 Effect of oral consumption of vitamin D on uterine fibroids: A randomized clinical trial. Complement Ther Clin Pract 2020, 39, 101159. doi: 10.1016/j.ctcp.2020.101159 and Hajhashemi et al. 2019 CJIM Line 45 and 49 You are using the association word once in Part I and then in PArt II. This might be misleading for the reader. Try to use the same word in each part.

	Page 6 and page 9 Major concerns I am not sure but this text look like you are mixing both IOM and Endocrine Society guideliness. 12 ng is from IOM and 21 -29 is from Endocrine. I think you should use only one guidelines. I don't know which were adapted by China. I found something like that in a paper by Wei et al. (2019) 10.1038/s41598-019-56297-y it looked like China was using the guidelines from USA. If you will decide which guidelines should be used. Then the second point is the supplementation. Why those doses? I cannot se any good explanation here. I would like a Table that will show the guidelines and present the chosen protocol. This will be much easier to understand for potential reader. This would be also better for the future so this could be repatable. I am giving you also some advice on my view on supplementation guidelines. Pludowski et al. 2018 10.1016/j.jsbmb.2017.01.021 (this is about the supplementation that covers also non-skeletal functions) There is some very good guideline about the use of vitamin D in Polish population 10.3389/fendo.2018.00246 by Rusińska et al. This is only for your information. You are using the reference by Holick et al. 2011 which also covers this topic, which is well known and really good. In my opinion it is necessary to fully highlight the choices of doses as this is a topic of major discussion these days. Also I think when you are mentioning the doses like 800IU you should add the number of tablets tyou would be giving to patietns (e.g. 800 IU - 2 tablets) just to be precise. Page 7 Inclusion 3 please explain that? I don't know why did you choose that values. Exclusion criteria. I think it is necesary to add the values of vitamin D that excludes patients even if they are mentioned above in inclusion. Inclusion 4 - cyst is also a kind of tumor. Will it be a exclusion criteria? Please be precise here. Exclusion 11 - Please rewrite. What does this mean if researcher thinks a subject is not suitable? Page 8 withdrawal 3 - please provide some cut of values. poor compliance can be like 90 and 1 % as well. Depends. The major question arises here. Authors did not provide the info what they will do in the situation where women will change the vitamin D deficiency into sufficiency or deficiency into insufficiency. These should be described here? Will those women be withdrawed? This is also to be decided by the ethical board if then if women will be sufficient can you still use the doses that are registered for insufficient or deficient etc. This info is of great importance. Page 10 There is no info about the patients with uterus myomatosus. In some cases those uteruses are so diffcicult to scan and the results are not of good quality. I think these should be mentioned somewhere. Like how many fibroids are max to scan or if this

	would be noted if patients will be withdrawn or excluded if their fibroids are not available for good quality scanning. Page 13 Finally on page 13 you mentioned the guidelines by the Osteoporosis from China, but they are still 12 - 20 and not 12 - 30. Maybe I am not reading this data in correct way, but still it is really confusing and in my opinion those guidelines are mixed in your manuscript. Once again major points - clearly which guidelines, and what doses (and why), figure or table to describe that. Figure 1 and 2 are wrong as you are testing women if they are deficient or not and then you are excluding them. And here it looks like you are excluding women at the beginning and then you are checking the VD serum level. Overall I think this study is really interesting and I hope it will generate some really good data. However there are some points to be edited or done little different before you can use this protocol. I hope my suggestions were helpful. I think also that this paper might require some quick double check with clinical trial manager and statistician to check if there are no minor mistakes. Best regards

VERSION 1 – AUTHOR RESPONSE

Reviewer #1:
The manuscript is subjected on a popular topic, Vitamin D, which is also proved to have influence on uterine leiomyomas. Previous studies have demonstrated that vitamin D was significantly lower in uterine leiomyomas. Yet, a well-designed prospective study on the preventive effect of vitamin D for uterine leiomyomas has not been published. This study design aims to evaluate this preventive effect of vitamin D.
Response: We appreciate the reviewer's positive and insightful comments for our research. Following these outstanding instructions, we have extensively modified our manuscript accordingly.
Comments:
1. The English language usage is appropriate, only few spelling errors needs to be corrected.
Response: We appreciate the reviewer for commending our work in English language usage of this manuscript. We have carefully checked the whole manuscript and corrected all spelling errors. In addition, the manuscript has also been revised by a native English speaker before resubmitting.
2. My major concern is that, vitamin D has an important role on many metabolic pathways and some group of patients will not be treated for a 2 years period. The ethical issue shall be re-considered.
Response: We greatly thank the reviewer for this excellent comment. It is known that the production of 1,25-dihydroxyvitamin D3 followed by its binding to vitamin D receptor (VDR) is responsible for triggering several ubiquitous metabolic actions, among which maintaining a tight calcium and phosphorus homeostasis in the circulation is the most important. We agree with that inappropriate use of vitamin D may lead to clinical symptoms of vitamin D toxicity (VDT), including hypercalcemia and hypercalciuria. However, these adverse effects are rare and usually caused by extremely excessive long-term intake of vitamin D. In 2011, An Endocrine Society Clinical Practice Guideline in America recommended that vitamin D3 at 2000 IU/day is the maintenance dose for adults diagnosed with

vitamin D deficiency after initial therapy. Another clinical trial from Lebanon showed that supplementation of vitamin D3 at 2000 IU/day for one year is safe as well as achieving adequate and optimal 25-hydroxyvitamin D concentrations. The Food and Nutrition Board of the Institute of Medicine provided evidences that vitamin D3 supplementation <10000 IU/day usually did not lead to VDT even for several years. Generally, we aim to achieve and maintain the optimal 25-hydroxyvitamin D concentrations with no adverse effects with vitamin D supplementation. According to the Endocrine Society guidelines from USA, patients with persistent serum 25-hydroxyvitamin D >150 ng/ml is regarded as suspected VDT, while it can be considered safe when serum 25-hydroxyvitamin D level is 100 ng/ml. Considering that the maximum dosage of vitamin D3 used in this study is 1600 IU/day in intervention group A from both Part I and II, we decide to monitor the serum 25-hydroxyvitamin D concentration regularly from the first use of vitamin D3. In this study, if someone's serum 25-hydroxyvitamin D level >150 ng/ml, stop taking vitamin D and treat hypercalcemia in time, and such a subject will be marked for withdrawal. We confirm that this issue has been included in ethics in our study protocol.

3. As far, I haven't noticed the patients' body mass index in the inclusion or exclusion criteria. Vitamin D levels also may be influenced by the BMI, so I would rather this criteria should be considered.

Response: We appreciate the reviewer for this insightful comment for our research. Vitamin D is fat soluble, and distributed into fat, muscle, liver, and serum. All of these compartments are increased in volume in obesity, resulting in a low vitamin D status in obese patients. Thus, higher doses supplementation of vitamin D is needed in obese people to achieve the same serum 25-hydroxyvitamin D level as normal weight. The Endocrine Society in the USA also recommended that a daily vitamin D dose for obese people (BMI >30 kg/m2) was set as "three times" greater than the recommended dose for subjects with normal body weight. We agree that vitamin D levels can also be influenced by the BMI. Therefore, the following item related to BMI has been added to the exclusion criteria: BMI <18.5 kg/m2 or BMI >25 kg/m2.

Reviewer #3:

Dear Authors

I have read your article with great interest. I am happy that the idea of vitamin D use in uterine fibroids is studied all over the world. I am happy with this trial as we need randomized controlled trials to found if we can treat fibroids with this compound.

Response: We thank the reviewer for the positive and insightful comments to guide us to further improve our manuscript.

Comments:

1. Abstract should be rewritten after the full review adding some new things from points discussed later (there is also no info about the method of measurement in abstract).

Response: We appreciate the reviewer for this good suggestion. We have rewritten the abstract section by adding descriptions about the measurement methods in the revised manuscript.

2. Another limitation (which is a strength some way). One population-only Chinese women.

Response: We greatly thank the reviewer for this reminding. Uterine fibroids are a worldwide disease. It differs between different generalizability. Therefore, it is indeed a limitation for implementing in only one hospital in Chinese subjects.

3. Page 4

Line 10: Not exactly, there are also groups of women with less incidence like 25% etc.

Response: We greatly thank the reviewer for this reminding. We have modified such description about the incidence of uterine leiomyomas in this sentence.

Line 15: As you did not add the per year info this is misleading with the sentence above. Please rewrite this part about the epidemiological data.

Response: We apologize for the missing about the per year info in this sentence. We have rewritten this sentence in the revised manuscript.

Line 25: UAE is not a method of choice and cannot be treated the same place like myomectomy and hysterectomy. You can mention it as a second line method (e.g. like in your reference Vilos et al. 2015)
Response: We appreciate the reviewer for this correction about treatment methods in uterine fibroids. We have corrected this sentence in the revised manuscript and mentioned UAE as a second line method.
Line 29: Please add examples of existing GnRH analogs, you can stay up to date with the new oral ones. Please provide the group for mifepristone, however I am not sure if this compound frequently used to treat fibroids. I would rather place SPRMs here, as they were used for some years with good overall results.
Response: We thank the reviewer for this excellent suggestion. We have provided one example of existing GnRH analogs 'leuprorelin' that has been approved for use in China. We have also added some descriptions about SPRMs here as they act as important progesterone receptor inhibitors. Actually, mifepristone is the first SPRM and we confirm that it has been approved for treating UFs in China.
4. Page 5
Line 10: I would add the recent data by Corachan et al. 2019 and 2020 about vitamin D and fibroids.
Response: We appreciate the reviewer for this excellent suggestion. We apologize for missing these two important studies published most recently. We have read them carefully and added the data about vitamin D and fibroids from them in the revised manuscript.
Line 12-15: I am not sure if you should add this info. This data is unpublished and there is plenty of data about this that could be directly read by an interested reader.
Response: We thank the reviewer for this kind suggestion. Indeed, this data described here had not been published when we submitted this manuscript on March 25th, 2020. Fortunately, these results by our group have been accepted on April 8th, 2020 and published on May 8th, 2020 in Journal of International Medical Research. Herein, we have added the citation of this study in the revised manuscript.
Line 25: Please check the very recent data by Arjeh et al. 2020.
Effect of oral consumption of vitamin D on uterine fibroids: A randomized clinical trial. Complement Ther Clin Pract 2020, 39, 101159. doi: 10.1016/j.ctcp.2020.101159
and Hajhashemi et al. 2019 CJIM
Response: We appreciate the reviewer for this good comment. Accordingly, we have added such description and cited this recent paper by Arjeh in the revised manuscript.
Line 45 and 49: You are using the association word once in Part I and then in Part II. This might be misleading for the reader. Try to use the same word in each part.
Response: We apologize for using the association word unproperly in two parts. We have changed the 'association' word in Part I into 'efficacy' and modified the 'efficacy' in Part II into 'association'.
5. Page 6 and page 9
I am not sure but this text look like you are mixing both IOM and Endocrine Society guidelines. 12 ng is from IOM and 21-29 is from Endocrine. I think you should use only one guideline. I don't know which were adapted by China. I found something like that in a paper by Wei et al. (2019) 10.1038/s41598-019-56297-y it looked like China was using the guidelines from USA.
Response: We appreciate the reviewer for this critical comment on which guideline was used to define vitamin D insufficiency and deficiency in this study. We confirm that the Endocrine Society Clinical Practice Guideline from USA was selected to be used in this study. According to this guideline, vitamin D deficiency is defined as a serum 25-hydroxyvitamin D3 level ≤20 ng/ml, and vitamin D insufficiency as a serum 25-hydroxyvitamin D3 level of 21-29 ng/ml. However, in China, patients who diagnosed with severe vitamin D deficiency (serum 25-hydroxyvitamin D3 level <12 ng/ml) must be sent to hospital for treatment and cannot be subjects to any clinical trial. Thus, women with very low serum 25-hydroxyvitamin D3 level (<12 ng/ml) will be excluded from this study.
If you will decide which guidelines should be use. Then the second point is the supplementation. Why those doses? I cannot see any good explanation here. I would like a Table that will show the

guidelines and present the chosen protocol. This will be much easier to understand for potential reader. This would be also better for the future so this could be repeatable.
I am giving you also some advice on my view on supplementation guidelines.
Pludowski et al. 2018 10.1016/j.jsbmb.2017.01.021 (this is about the supplementation that covers also non-skeletal functions)
There is some very good guideline about the use of vitamin D in Polish population 10.3389/fendo.2018.00246 by Rusinska et al. This is only for your information.
You are using the reference by Holick et al. 2011 which also covers this topic, which is well known and really good. In my opinion it is necessary to fully highlight the choices of doses as this is a topic of major discussion these days.
Also I think when you are mentioning the doses like 800 IU you should add the number of tablets that you would be giving to patients (e.g. 800 IU-2 tablets) just to be precise.
Response: We appreciate the reviewer for this excellent suggestion. We have decided to use the Endocrine Society Clinical Practice Guideline from USA in this study. According to this guideline, vitamin D deficiency is defined as a serum 25-hydroxyvitamin D3 level <20 ng/ml, insufficiency as 21-29 ng/ml, and sufficiency as at least 30 ng/ml to maximize bone health. Based on this definition, this guideline suggests that adults at risk for vitamin D deficiency (aged 19-20 years) require at least 1500-2000 IU/day of vitamin D to raise the blood level of 25-hydroxyvitamin D3 consistently above 30 ng/ml. For an adult diagnosed with vitamin D deficiency, 50,000 IU/week of vitamin D3 for 8 weeks (equivalent to approximately 7000 IU/day) are needed to achieve a blood level of 25-hydroxyvitamin D3 above 30 ng/ml. After 8 weeks, these adults should also receive additional maintenance therapy of 1500-2000 IU/day. Previous studies also indicate that a vitamin D deficient patient with a serum 25-hydroxyvitamin D3 level reaching approximately 20 ng/ml requires 100 IU of vitamin D to raise blood levels of 25-hydroxyvitamin D3 by 0.6-1 ng/ml. Therefore, we decide to select 1600 IU (4 capsules)/day and 800 IU (2 capsules)/day to treat subjects with vitamin D deficiency and insufficiency respectively, which are adequate and effective for these subjects. Indeed, the aim of vitamin D supplementation is to achieve and maintain the optimal 25-hydroxyvitamin D3 levels with no adverse effects. Pietras et al. reported that supplementation of vitamin D at 50,000 IU/2 weeks (equivalent to approximately 3300 IU/day) for up to 6 years is safe and adequate even for healthy adults to maintain serum 25-hydroxyvitamin D3 concentration in the desired range of 40-60 ng/ml, with no evidence of vitamin D toxicity (VDT). Consistently, Ekwaru et al. also showed that healthy adults from Canada had a significant increase of serum 25-hydroxyvitamin D3 level up to 60 ng/ml after vitamin D intake at 20,000 IU/day, but without any evidence of VDT. According to the Endocrine Society guidelines from USA, patients with persistent serum 25-hydroxyvitamin D >150 ng/ml is regarded as suspected VDT, while it can be considered safe when serum 25-hydroxyvitamin D level is 100 ng/ml. Thus, supplementation of vitamin D at 1600 IU/day for 800 IU/day for 2 years is almost safe. In addition, we decide to monitor the serum 25-hydroxyvitamin D3 concentration regularly from the first use of vitamin D3. In this study, if someone's serum 25-hydroxyvitamin D level >150 ng/ml, stop taking vitamin D and treat hypercalcemia in time, and such a subject will be marked for withdrawal. We have also added the number of capsules that we would give to patients in the revised manuscript. We have also listed several important clinical trials published recently related to vitamin D use in a new depicted Table 1 in the revised manuscript, to obtain a better understanding of our protocol for readers.
6. Page 7
Inclusion 3: Please explain that? I don't know why did you choose that values.
Response: We thank the reviewer for pointing out this question. According to the Endocrine Society Clinical Practice Guideline, vitamin D deficiency is defined as a serum 25-hydroxyvitamin D3 level ≤20 ng/ml, and vitamin D insufficiency as a serum 25-hydroxyvitamin D3 level of 21-29 ng/ml. However, in China, patients who diagnosed with severe vitamin D deficiency (serum 25-hydroxyvitamin D3 level <12 ng/ml) must be sent to hospital for treatment and cannot be subjects to any clinical trial. Thus, we made the inclusion criteria 3 that women with serum 25-hydroxyvitamin D3 level of 12-29 ng/ml will be enrolled in this trial.

Exclusion criteria: I think it is necessary to add the values of vitamin D that excludes patients even if they are mentioned above in inclusion.
Response: We thank the reviewer for this good advice. We have added the values of vitamin D that excludes subjects as the exclusion criteria 1 in both part I and II.
Inclusion 4: cyst is also a kind of tumor. Will it be an exclusion criteria? Please be precise here.
Response: We thank the reviewer for this good advice and apologize for not explaining clearly. We know that most of ovarian cysts are a physiological phenomenon. They will disappear as the menstrual cycle progresses. Hence, we would not exclude them. The other pathological ovarian cyst is also a kind of tumor. We will be excluded following “Exclusion criteria: Suspected or identified as other tumors of genital tract”.
Exclusion 11: Please rewrite. What does this mean if researcher thinks a subject is not suitable?
Response: We apologize for adding this criteria which is unclear. We have deleted this part in the revised manuscript.
7. Page 8
Withdrawal 3: Please provide some cut of values. Poor compliance can be like 90 and 1% as well. Depends.
Response: We appreciate the reviewer for this good suggestion. We have redefined the withdrawal criteria 3 that patients with poor compliance: Actual oral dose/dose *100% ≤80% or Actual oral dose/dose*100% ≥120%. We have displayed this part in the revised manuscript.
The major question arises here. Authors did not provide the info what they will do in the situation where women will change the vitamin D deficiency into sufficiency or deficiency into insufficiency. These should be described here? Will those women be withdrawn? This is also to be decided by the ethical board if then women will be sufficient can you still use the doses that are registered for insufficient or deficient etc. This info is of great importance.
Response: We thank the reviewer for pointing out this critical comment. The Endocrine Society Guideline from USA suggests that adults at risk for vitamin D deficiency require at least 1500-2000 IU/day of vitamin D. For a vitamin D deficient adult, 50,000 IU/week of vitamin D3 for 8 weeks (equivalent to approximately 7000 IU/day) are required followed by additional maintenance therapy of 1500-2000 IU/day. Even in healthy adults, supplementation of vitamin D at 50,000 IU/2 weeks (equivalent to approximately 3300 IU/day) for up to 6 years is also effective and safe to maintain serum 25-hydroxyvitamin D3 concentration in the desired range of 40-60 ng/ml, with no evidence of VDT. Moreover, as shown in Table 1, 2000 IU/day of vitamin D are the most widely used doses in most of important clinical trials published recently, with no evidence of VDT reported. Despite not used in our study, the vitamin D supplementation principles from the Polish Society recommended adults with serum 25-hydroxyvitamin D3 concentration >20-30 ng/ml, to start vitamin D intake at doses suggested for peers from the general population (800-2000 IU/day) if vitamin D was not provided previously. Therefore, in our study, all subjects in each group will receive the same doses of vitamin D persistently during the whole clinical trial. Furthermore, women will not be withdrawn if they change the vitamin D deficiency into sufficiency or deficiency into insufficiency. We also confirm that this decision has been approved by the ethical committee of our hospital before starting the trial.
8. Page 10
There is no info about the patients with uterus myomatosus. In some cases, those uteruses are so difficult to scan and the results are not of good quality. I think these should be mentioned somewhere. Like how many fibroids are max to scan or if this would be noted if patients will be withdrawn or excluded if their fibroids are not available for good quality scanning.
Response: We appreciate the reviewer for this great comment. We have modified this part and add “some cases those uteruses are difficult to scan or the amount of UFs is more than 4” to the exclusion criteria. Meanwhile, we have deleted “researcher thinks a subject is not suitable”.
9. Page 13
Finally, on page 13 you mentioned the guidelines by the Osteoporosis from China, but they are still 12-20 and not 12-30. Maybe I am not reading this data in correct way, but still it is really confusing and in my opinion those guidelines are mixed in your manuscript.

Response: We appreciate the reviewer for pointing out this issue. Actually, we just discussed and compared several different guidelines about vitamin D intake based on serum level. We have confirmed that the Endocrine Society Clinical Practice Guideline from USA was selected to be used in this study. According to this guideline and the special condition (women with serum 25-hydroxyvitamin D3 level <12 ng/ml cannot be enrolled in any clinical trial) in China, women with 25-hydroxyvitamin D3 level of 12-29 ng/ml will be selected as subjects in our trial.

10. Once again major points: Clearly which guidelines, and what doses (and why), figure or table to describe that.

Response: We greatly thank the reviewer for this kind reminding on this issue. As described above, we have confirmed the guideline use and illustrated doses selection and reasons in our study. We have also depicted a Table 1 that lists the regimen of vitamin D from recently published clinical trials.

11. Figure 1 and 2 are wrong as you are testing women if they are deficient or not and then you are excluding them. And here it looks like you are excluding women at the beginning and then you are checking the VD serum level.

Response: Thank the reviewer for this advice. We have modified Figure 1 and 2 in the manuscript.

12. Overall I think this study is really interesting and I hope it will generate some really good data. However, there are some points to be edited or done little different before you can use this protocol. I hope my suggestions were helpful.

Response: We greatly thank the reviewer for pointing out these positive and helpful comments to our manuscript. We have modified our manuscript accordingly and now the manuscript has been improved a lot indeed.

13. I think also that this paper might require some quick double check with clinical trial manager and statistician to check if there are no minor mistakes.

Response: We thank the reviewer for this kind reminding. This paper has received a quick double check with clinical trial manager and statistician by a specialist on clinical trial management. We confirm that there are no major and minor mistakes in the revised manuscript.