OPEN PEER REVIEW

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As a new feature for progressing towards transparency, we decided to open a new window for all of our editors as well as authors titled "**Open Peer Review**". We hope by this new facility, our reviewers will be more motivated and authors will be more satisfied with the review process. We believe that publishing our peer review reports could make a transparent and clear environment for all our efforts within a journal, but not all reviewers tend to publish their comments.

What is "Open Peer Review" process?

An "Open Peer Review" process is making the details of all review process (including reviewers, associate editors, and EICs comments) as "Public" as it is agreed by EIC, Authors, and reviewers.

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- More motivations for all involved roles in the review process
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- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

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Iranian Journal of Psychiatry and Behavioral Sciences
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Peer Review Report for "The Effects of Vitamin D Supplementation on Withdrawal Symptoms and the Expression of Inflammatory Cytokines and Insulin in Patients Under Methadone Maintenance Treatment: A Randomized, Double-Blind, Placebo-Controlled Trial"

Author(s): Amir Ghaderi, Hamidreza Banafshe, Esmat Aghadavod, Mostafa Gholami, Zatollah Asemi, Azam Mesdaghinia

Review Timeline:

Submit Date:	1 Dec 2018
Revised Date:	14 Dec 2019
Accepted Date:	18 Jan 2020

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (0) 11 Dec 2018

Dear AE,
This manuscript describes Clinical and genetic response to vitamin D supplementation in methadone maintenance treatment subjects. This is a valuable topic and very interesting. I have a few minor points that should be addressed before acceptance.
1. I think the title would be more appropriate. The title is not attractive. Modify as follows: Clinical trial of the effects of vitamin D supplementation on withdrawal symptoms and gene expression related to inflammatory cytokines and insulin in methadone maintenance treatment subjects
2. In Introduction section: please refer to the epidemiological of the prevalence of drug use and methadone consumption.

3. In section Introduction, Line 24 should “their” be “its”?
4. Were all participants men? No mention of gender is ever made. If only men, please explain. If there were women as well, please show your results and main Tables stratified by gender.
5. Is there a precise documentation of side effects following international standards? Please provide this information.
6. Please note that in past studies, patients with MMT have inflammatory and oxidative stress?
7. Please note that vitamin D is effective in patients under MMT.
Thanks

REFEREE: Reviewer | **Revision** (0) 13 Dec 2018

Dear AE,
I have read this manuscript with interest. This is a manuscript where the authors examined the Clinical and genetic response to vitamin D supplementation in methadone maintenance treatment subjects. The manuscript was interesting and can be much improved if the following issues are briefly discussed:
1. The selected concentrations of vitamin D used in this study should be supported with appropriate references.
2. Please explain more about the implication of this study results. How can the study results help clinically?
3. Did not side effects be seen in patients? Please explain.
4. Please indicate how many patients were at the beginning of the study. How many people were enrolled in the study and how many were excluded. Please explain.
5. Please give reference for these sentences:
“Several studies have shown that hypovitaminosis D may be important in addiction”
6. Patients received methadone syrup or methadone tablets?
7. Was the design double-blinded or single-blinded?
8. At the end of the discussion, study limitations should be noted.
9. Generally the grammar needs some work to bring up it to standard but could be achievable.
Thanks.

REFEREE: Reviewer | **Revision** (0) 21 Dec 2018

Dear AE,
The authors investigated Clinical and genetic responses to vitamin D supplementation in methadone maintenance treatment subjects. For the aim, the authors used 40 MMT subjects. The authors observed the protective role of vitamin D supplementation on Clinical and genetic responses. This paper is novel due to the population studied and some significant results are reported which add to the field. However, I have a few points that should be addressed by the authors in the revised version.
1. Patients of the present study undergoing methadone maintenance treatment, it is recommended that Text changed subjects to patients.
2. What was the justification for your choice of vitamin D dose?
3. The number of samples in the sample size and Results do not match each other. This

needs checking.
4. As patients of this trial are undergoing methadone maintenance treatment, it will be interesting that data on the type of agonist (syrup or tablet) for both groups add to the Results section.
5. I suggest that the prevalence and significance of vitamin D deficiency in MMT should be discussed in Discussion section.
Thanks

REFEREE: Reviewer | **Revision** (0) 4 Jan 2019

Dear AE,
Clinical and genetic response to vitamin D supplementation in methadone maintenance treatment subjects
Summary:
In this study, the impact of vitamin D intake on withdrawal symptoms and genetic response related to inflammatory cytokines and insulin in MMT subjects was investigated. A randomized, double-blind, placebo-controlled trial was conducted for 40 people under MMT to receive either 50,000 IU vitamin D (n=20) and placebo (n=20) every 2 weeks for 3 months. According to their obtained results, vitamin D intake for 3 months in MMT patients significantly ameliorated IL-1 and PPAR-γ expression, but did not impact COWS and IL-8 and TNF-α expression.
Comments:
My observations on the manuscript in the order of their appearance may be stated as follows:
1. Line 24-25: Is MMT favorable for any kind of drug dependence like Benzodiazepines? Or it is better to say: MMT is favorable for treatment of opioid dependence. (Not drug dependence)?
2. Line 49: From the last paragraph of the Introduction, as well as from the abstract, method, discussion and conclusions, the significance of the contribution of the current study is not clear. The manuscript needs to be improved to demonstrate its contribution.
3. Line 117-118: As mentioned in this part of manuscript: " the favorable effects of vitamin D intake for 3 months on withdrawal symptoms and gene expression associated with inflammatory cytokines and insulin subjects under MMT"? As it is clear, when the patients are under MMT, they do not experience withdrawal symptoms. When subjects in this study discontinue the methadone and experience the withdrawal symptoms? More detailed explanations have to be presented in this part of manuscript to clarify.
4. Line 125: according to ref. [14] it is better to change the sentences as follows:
MMT is led to some disorders, including increased inflammatory cytokines.
5. Some grammatical and typesetting errors observed in the manuscript. For example Line 138: "The same as findings were observed by others [20-21]" must be changed to "The same findings were observed by others [20-21]". …
Conclusion:
Mainly due to my comment 3 above, I believe that a major revision is required for this manuscript to be published in the Iranian Journal of Psychiatry and Behavioral Sciences.
Thanks.

REFEREE: Associate Editor | **Revision** (0) 8 Feb 2019

Dear EIC,
Many thanks for submitting" Clinical and genetic response to vitamin D supplementation in methadone maintenance treatment subjects". I received comments from reviewers who have expertized in the manuscript. Although the reviewers found considerable strengths in this paper, they also identified some concerns and believed that the paper needs some revisions. I conclude due to the importance of the subject, the manuscript has the potential of being considered for publication after satisfactorily addressing the reviewers' concerns. Therefore, I invite the authors to reply to the reviewers' comments and resubmit their revised manuscript for further review. If they choose to revise the submission please upload the revised manuscript and an annotated list addressing the reviewers' comments and highlighting where changes have been made to the paper.
Best regards

REFEREE: EIC | **Revision** (0) 15 Feb 2019

Dear Authors,
Your paper has been evaluated by external peer reviewers. Please do your revisions according to the reviewers' comments. I appreciate you doing the revisions as soon as possible by using the track changes mode in MS Word and using colored text.
After revision authors are obliged to upload reviewers' comments word file in which they have replied to all comments one by one.
Besides, please pay attention to the word count limitation:
Word count limits are declared in this link: http://kowsarpub.com/en/index.php/services/for-authors/895-word-count
Thanks.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Dr Mehran Zarghami,

Thank you very much for your letter informing us of your decision regarding the manuscript no. **ID- 86969** entitled **“Clinical and genetic response to vitamin D supplementation in methadone maintenance treatment subjects**". The manuscript has been revised according to the referees’ comments. Responses to the reviewers' comments have been provided below.

Yours Sincerely,

Associate Editor's Comments:

Many thanks for submitting" Clinical and genetic response to vitamin D supplementation in methadone maintenance treatment subjects". I received comments from reviewers have expertized on the manuscript. Although the reviewers found considerable strengths in your paper, they also identified some concerns and believed that the paper needs some revisions. I conclude due to the importance of the subject, the manuscript has the potential of consideration for publication after satisfactorily addressing reviewers concerns. Therefore, I invite you to reply to the reviewers comments and resubmit your revised manuscript for further review. If you choose to revise your submission please upload your revised manuscript and an annotated list addressing the reviewers comments and highlighting where changes have been made to the paper. Authors: Thank you. First of all, we would like to thank the reviewers for their great comments to improve the quality of the manuscript. We tried our best to revise the manuscript according to the reviewers' comments.

Reviewer 1:

This manuscript describes Clinical and genetic response to vitamin D supplementation in methadone maintenance treatment subjects. This is a valuable topic and very interesting. I have a few minor points that should be addressed before acceptance.

1. I think the title would be more appropriate. The title is not attractive. Modify as follows: Clinical trial of the effects of vitamin D supplementation on withdrawal symptoms and gene expression related to inflammatory cytokines and insulin in methadone maintenance treatment subjects

Authors: This point was done:

“Clinical trial of the effects of vitamin D supplementation on withdrawal symptoms and gene expression related to inflammatory cytokines and insulin in methadone maintenance treatment patients” (Lines 1-3).

2. In Introduction section: please refer to the epidemiological of the prevalence of drug use and methadone consumption.

Authors: This point clarified:

“In Iran, the prevalence of opioid use is rising and was nearly three times higher than the prevalence worldwide. About 1.2 millions of Iranians have opioid dependency [2]. About 500,000 people are under buprenorphine and methadone treatments [3]” (Lines 25-8).

3. In section Introduction, Line 24 should “their” be “its”?

Authors: This point was done.

4. Were all participants men? No mention of gender is ever made. If only men, please explain. If there were women as well, please show your results and main Tables stratified by gender.

Authors: Yes. This point clarified:

“At baseline, male patients were randomised …..” (Line 76).

5. Is there a precise documentation of side effects following international standards? Please provide this information.

Authors: Thank you for this important point. This comment added to the revised version:

“No serious adverse effects were reported following the taking of vitamin D in patients” (Lines 114).

6. Please note that in past studies, patients with MMT have inflammatory and oxidative stress?

Authors: This point clarified:

"Drug abuse (i.e., opioids and methadone) would result in increased inflammatory parameters and oxidative stress . *In vitro* and *in vivo* medical evidence have reported that opioid dependence has complication immunomodulatory impacts on adaptive immune responses and inflammatory markers . Increased gene expression of inflammatory cytokines has been evaluated in noradrenergic locus coeruleus cells in the brains of opioid dependence patients " (Lines 28-33).

7. Please note that vitamin D is effective in patients under MMT.

Authors: Thank you for this important point. This comment added to the revised version:

"Recently, the issue of vitamin D administration is suggested in patients under MMT. This may be due to the favorable effects of vitamin D in MMT subjects and diseases associated with metabolic disorders. We have previous studies demonstrated that prescribe a pharmacologic dose of vitamin D (50,000 IU/2-week) for 3 months in MMT people improved mental health scales and metabolic outcomes [9]" (Lines 37-41).

Reviewer 2:

I have read your manuscript with interest. This is a manuscript where the authors examined the Clinical and genetic response to vitamin D supplementation in methadone maintenance treatment

subjects. The manuscript was interesting and can be much improved if the following issues are briefly discussed.

1. The selected concentrations of vitamin D used in this study should be supported with appropriate references

Authors: Thank you. This point added to the revised file:

“he lack of information about the suitable vitamin D dosage for subjects with MMT led to we applied the above-considered dose of vitamin D based on a previous report in chronic liver disorder cases [20]” (Lines 79-81).

2. Please explain more about the implication of this study results. How can the study results help clinically?

Authors: This point has been clarified in the revised version.

“Vitamin D can be recommended as an adjunct to MMT in opioid withdrawal a protocol which may elevate quality of life and may diminish methadone adverse effects” (Lines 197-99).

3. Did not side effects be seen in patients? Please explain

Authors: Thank you for this important point. This comment added to the revised version:

“No serious adverse effects were reported following the taking of vitamin D in patients” (Lines 114).

4. Please indicate how many patients were at the beginning of the study. How many people were enrolled in the study and how many were excluded. Please explain.

Authors: Thank you. This point was done:

“This randomized double-blind placebo-controlled clinical trial, registered in the Iranian website for registration of clinical trials as http://www.irct.ir, no: IRCT2017042433551N1, was performed in 40 MMT people, aged 25-70 y referred to the Golabchi Clinic, from February 2017

to June 2017. Ethical approval for this study was IR.Kaums.REC.1395.118. In this investigation, informed consent was taken from all people. Exclusion criteria were: using colecalciferol 3 months before the intervention, and people with history of metabolic diseases, including diabetes, cardiovascular and hypertension" (Lines 67-3).

5. Please give reference for these sentences:

"Several studies have shown that hypovitaminosis D may be important in addiction"

Authors: This comment has been addressed:

"Several studies have shown that hypovitaminosis D may be important in addiction, and may have an effect in destabilizing serotoninergic, dopaminergic and glutamatergic pathways, which subsequently contribute to the degenerating mental state [29, 30]" (Lines 150-3).

6. Patients received methadone syrup or methadone tablets??

Authors: This comment has been addressed:

"The used form of methadone was received as syrup by subjects" (Lines 81).

7. was the design double-blinded or single-blinded?

Authors: Yes. This point clarified:

"This randomized double-blind placebo-controlled clinical trial, registered in the Iranian website ….." (Line 67).

8. At the end of the discussion, study limitations should be noted.

Authors: This point has been added to the revised file:

"The present study had some limitations. Firstly, duration of this study was short. Long-term duration may be lead to better effects in withdrawal symptoms. Secondly, we did not evaluate the effects of colecalciferol on cognitive function. Thirdly, we could not evaluate the acute and chronic pain in MMT patients " (Lines 191-4).

9. Generally the grammar needs some work to bring up it to standard but could be achievable.

Authors: Paper edited by a native speaker.

Reviewer 3:

The authors investigated Clinical and genetic response to vitamin D supplementation in methadone maintenance treatment subjects. For the aim, the authors used 40 MMT subjects. The authors observed protective role of vitamin D supplementation on Clinical and genetic response.

This paper is novel due to the population studied and some significant results are reported which add to the field. However, I have few points that should be addressed in the revised version

1. Patients of the present study undergoing methadone maintenance treatment, it is recommended that in Text changed subjects to patients.

Authors: This point was done.

2. What was the justification in your choice of vitamin D dose?

Authors: Thank you. This point added to the revised file:

"The lack of information about the suitable vitamin D dosage for subjects with MMT led to we applied the above-considered dose of vitamin D based on a previous reports in chronic liver disorder cases [20]" (Lines 79-1).

3. The number of samples in the sample size and Results do not match with each other. This needs checking.

Authors: I apologize for this mistake. This point has been corrected.

4. As patients of this trial are undergoing methadone maintenance treatment, it will be interesting that data on the type of agonist (syrup or tablet) for both groups add to Results section.

Authors: This comment has been addressed:

"The used form of methadone was received as syrup by subjects" (Lines 81).

5. I suggest that the prevalence and significance of vitamin D deficiency in MMT should be discussed in Discussion section.

Authors: Thank you for this important comment. This point added to the revised version as follows:

“The number of studies have documented that hypovitaminosis D and BMD are a difficult in MMT patients [26, 27]. In a study of subjects with chronic pain, 93% of them had colecalciferol deficiency [28]” (Lines 145-7).

Reviewer 4:

Clinical and genetic response to vitamin D supplementation in methadone maintenance treatment subjects

Summary:

In this study, the impact of vitamin D intake on withdrawal symptoms and genetic response related to inflammatory cytokines and insulin in MMT subjects was investigated. A randomized, double-blind, placebo-controlled trial was conducted for 40 people under MMT to receive either 50,000 IU vitamin D (n=20) and placebo (n=20) every 2 weeks for 3 months. According to their obtained results, vitamin D intake for 3 months in MMT patients significantly ameliorated IL-1 and PPAR-γ expression, but did not impact COWS and IL-8 and TNF-α expression

1. Line 24-25: Is MMT favorable for any kind of drug dependence like Benzodiazepines? Or it is better to say: MMT is favorable for treatment of opioid dependence. (Not drug dependence)?

Authors: Yes. This point was done.

“Methadone maintenance therapy (MMT) is favorable for treatment of opioid dependence [1]” (Line 25).

2. Line 49: From the last paragraph of the Introduction, as well as from the abstract, method, discussion and conclusions, the significance of the contribution of the current study is not clear. The manuscript needs to be improved to demonstrate its contribution.

Authors: Authors: Thank you. This point added to the revised file as follows:

“Vitamin D may result in improved mental health symptoms, metabolic profiles, biomarkers of oxidative stress and inflammation through their effect on central nervous system, neuronal circuits and the regulation of pro-inflammatory cytokines in cellular systems [15-17] and through affecting signaling pathway and gene expression [18]. In addition, vitamin D treated animals of methamphetamine induced have demonstrated that the oral taking of a vitamin D can decrease in

metabolites, protection the dopaminergic system against the dopamine and serotonin depleting [19]. Although the impacts of consuming vitamin D on psychological outcomes have considered meaningful attention, data about gene expression related to inflammatory cytokines and insulin metabolism in MMT subjects are limited and inconsistent. The current study was, therefore, this study was aimed to evaluate the effects of colecalciferol intake on gene expression involved inflammatory cytokines and insulin metabolism in MMT patients" (Lines 53-63).

3. Line 117-118: As mentioned in this part of manuscript: "the favorable effects of vitamin D intake for 3 months on withdrawal symptoms and gene expression associated with inflammatory cytokines and insulin subjects under MMT"? As it is clear, when the patients are under MMT, they do not experience withdrawal symptoms. When subjects in this study discontinue the methadone and experience the withdrawal symptoms? More detailed explanations have to be presented in this part of manuscript to clarify.

Authors: Authors: Thank you. This point added to the revised file:

"It must be kept in mind that a number of studies have shown that patients treated with methadone are experienced withdrawal symptoms, that the onset of withdrawal syndrome lasts from 7 to 10 days and these symptoms continue up to few weeks [23, 24]. The most reason for successful termination treatment MMT was the belief that they needed the higher methadone dose and has experiences the withdrawal symptoms. However, we believe that these symptoms are not common in long term; further studies are needed to confirm our findings" (Lines 137-43).

4. Line 125: according to ref. [14] it is better to change the sentences as follows: MMT is led to some disorders, including increased inflammatory cytokines.

Authors: Thank you. This point clarified in the revised version.

“MMT is led to some disorders, including increased inflammatory cytokines [25]” (Lines 145).

5. Some grammatical and typesetting errors observed in the manuscript. For example, Line 138: “The same as findings were observed by others [20-21]” must be changed to “The same findings were observed by others [20-21]”. …

Authors: Thank you. This point was done and paper edited by a native speaker.

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (1) 11 Mar 2019

Dear AE,
The study is reviewed carefully. According to comments and revisions by the author, this manuscript is accepted.
Thanks

REFEREE: Reviewer | **Revision** (1) 12 Mar 2019

Dear Editor;
The requested changes are well revised and I can accept the present study in its current form.
Best,

REFEREE: Reviewer | **Revision** (1) 13 Mar 2019

Dear AE,
The study is reviewed carefully. According to comments and revisions by the author, this manuscript is accepted.
Thanks

REFEREE: Reviewer | **Revision** (1) 23 Mar 2019

Dear AE,
The answers of authors to my comments are reasonable and acceptable.
Thanks.

REFEREE: Reviewer | **Revision** (1) 13 Sep 2019

Dear AE
Thanks for revising the paper. Some of the necessary revisions have been done, but, I think the paper needs some other revisions as below:
1- Abstract should be structured as: background, objective, material/patients and methods, results and conclusion. This structure has not been considered in the abstract. It seems the first sentence of the "objective" could move to the " background ".
2- In the introduction there is some data about the decrease of inflammatory response and

cytokines due to vit D, but in the abstract you described the impact of hypervitaminosis D on some metabolic vulnerable, including metabolic disorders associated with inflammatory cytokines and insulin resistance in subjects undergoing methadone maintenance treatment. I think this part needs to be clarified.
3- Some abbreviations used in the manuscript without description.
4- Time and place of the study, statistical tests and p value should be mentioned in the abstract.
5- In the abstract, results were repeated in "conclusion".
6- Some key words are not selected from MESH.
7- Objective and conclusion should be mentioned as separated parts in the article body.
8- What was the reason of selection of the male subjects?
9- Sample size calculation should be clarified.
10- Reliability and validity of the questionnaire for Iranian population should be described.
11- The method of psychiatric comorbidity evaluation should be clarified.
12- The effect of vit D on withdrawal symptoms were evaluated, but there is not any explanation about methadone dose changes in the subjects in the study duration.
13- The implication of the results of genetic part of the study should be clarified more exactly.
14- English writing needs to be improved.
My decision: revision
Best regards

REFEREE: Associate Editor | **Revision (1)** 13 Sep 2019

Dear EIC,
The manuscript is studied, and there are some comments which should be addressed by the author. According to the comments, the document needs major revision.
Kind regards,

REFEREE: EIC | **Revision (1)** 20 Sep 2019

Dear Authors,
Thank you for your recent revisions.
Your paper has now been evaluated again. According to the reviewers' and associate editor's comments, it needs some more revisions. Please do your revisions according to these comments. I appreciate you doing the revisions as soon as possible by using the track changes mode in MS Word and using colored text.
Besides, please pay attention to the word count limitation.
Word count limits are declared in this link: http://kowsarpub.com/en/index.php/services/for-authors/895-word-count
We look forward to receiving your revised manuscript. Thank you for sending your work to the Iranian Journal of Psychiatry and Behavioral Sciences.
Thanks.

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Dr Mehran Zarghami,

Thank you very much for your letter informing us of your decision regarding the manuscript no. ID-86969 entitled "**Clinical trial of the effects of vitamin D supplementation on withdrawal symptoms and gene expression related to inflammatory cytokines and insulin in methadone maintenance treatment patients**". The manuscript has been revised according to the referees' comments. Responses to the editor's comments have been provided below.

Yours Sincerely,

Dear author

Thank you for revising the paper. Some of the necessary revisions have been done, but, I think the paper needs some other revisions as below:

Authors: Thank you. First of all, we would like to thank the reviewers for their great comments to improve the quality of the manuscript. We tried our best to revise the manuscript according to the editor's comments.

1. Abstract should be structured as: background, objective, material/patients and methods, results and conclusion. This structure has not been considered in the abstract. It seems the first sentence of the "objective" could move to the " background ".

Authors: This point was done:

*"**Background:*** Hypovitaminosis D may be related to some metabolic vulnerable, including metabolic disorders associated with inflammatory cytokines and insulin resistance in subjects undergoing methadone maintenance treatment (MMT).

Objective: This study determined the impact of vitamin D intake on withdrawal symptoms and genetic response related to inflammatory cytokines and insulin in MMT patients" (line 5-9).

2. In the introduction there is some data about the decrease of inflammatory response and cytokines due to vit D, but in the abstract you described the impact of hypervitaminosis D on some metabolic vulnerable, including metabolic disorders associated with inflammatory cytokines and insulin resistance in subjects undergoing methadone maintenance treatment. I think this part needs to be clarified.

Authors: I apologize for the confusion. This point has been corrected.

"Hypovitaminosis D may be related to some metabolic vulnerable, including metabolic disorders associated with inflammatory cytokines and insulin resistance in subjects undergoing methadone maintenance treatment (MMT)" (Line 5-7).

3. Some abbreviations used in the manuscript without description.

Authors: Yes. This point was done.

4. Time and place of the study, statistical tests and p value should be mentioned in the abstract.

Authors: Authors: Thank you. This point added to the revised file as follows:

"This investigation was done among 40 people under MMT to consume either 50,000 IU/2 weeks colecalciferol or placebo (n=20 each arm), between April and June 2017 in Kashan, Iran. The clinical opiate withdrawal symptoms (COWS) to examine withdrawal scale and gene expression were evaluated at baseline and after the 3 months' intervention. Statistical analysis of the data was performed using SPSS 17 with Fisher's exact test, t-test, chi-square and ANCOVA. P values <0.05 were considered statistically significant". (line 10-15).

5. In the abstract, results were repeated in "conclusion".

Authors: Authors: Thank you. This point added to the revised file.

*"**Results:*** Results of our study have been shown that compared with the placebo, taking vitamin D downregulated interleukin-1 (IL-1) expression (P=0.01) in Peripheral Blood Mononuclear Cells (PBMCs) of MMT patients. In addition, consuming vitamin D increased peroxisome proliferator-activated receptor gamma (PPAR-γ) expression (P=0.01). Administration of vitamin D unchanged COWS and IL-8 and tumor necrosis factor alpha (TNF-α) expression.

Conclusion: According to the results of the present study, vitamin D can be recommended as an adjunct to MMT which may elevate quality of life and decrease methadone side effects" (Line 16-22).

6. Some key words are not selected from MESH.

Authors: Thank you for this important point. As the reviewer pointed out some of the keywords are not Mesh and are considered as simple terms. Therefore, all keywords are revised now according to the Mesh terms as the reviewer suggested.

"Keywords: Vitamin D, inflammatory cytokines, Insulin, Methadone, Substance Withdrawal Syndrome" (Line 23-24).

7. Objective and conclusion should be mentioned as separated parts in the article body.

Authors: This point was done. This comment added to the revised version.

"Objectives: Due to the importance of vitamin D, this study aimed at examining the vitamin D supplementation on withdrawal symptoms and genetic response in patients who underwent MMT". (Line 66-69).

8. What was the reason of selection of the male subjects?

Authors: Thank you. We did not exclude the paricipants according to their sex. However, as the patients referring to the MMT center are mostly males, all participants included in the present trial were males.

9. Sample size calculation should be clarified.

Authors: This point was done.

3.6. Sample size

"In this study, we used a randomized clinical trial sample size calculation formula where type one (α) and type two errors (β) were 0.05, and 0.20 (power=80%), respectively. According to our previously published trial (18), we used 0.15 fold changes as the SD and 0.15 fold change as the change in mean (d) of TNF-α as a primary outcome. Based on the formula, we needed 16

patients in each group. After allowing for 20% dropouts in each group, the final sample size was 20 persons in each group". (Line 114-120).

10. Reliability and validity of the questionnaire for Iranian population should be described.

Authors: This point has been addressed:

"The Clinical Opiate Withdrawal Scale (COWS) which have been demonstrate to be reliable and valid measures for evaluating withdrawal signs in the withdrawal syndrome (21, 22). Nazari, et al., approved the validity and reliability of Persian version of COWS among Iranian population with opiate withdrawal syndrome (23) " (line 94-97).

11. The method of psychiatric comorbidity evaluation should be clarified.

Authors: This point clarified:

"The psychiatric comorbidity assessment was performed by medical records that had been examined previously by a psychiatrist".

12. The effect of vit D on withdrawal symptoms were evaluated, but there is not any explanation about methadone dose changes in the subjects in the study duration.

Authors: This point clarified:

Patients received a fixed dose of methadone during the intervention, so no dose changes were observed in this study. Also, there was no significant difference between the two groups in methadone dose and duration of methadone use. Please see table 2.

13. The implication of the results of genetic part of the study should be clarified more exactly.

Authors: This point was done. This comment added to the revised version.

"Pro-inflammatory markers demonstrated that directly oppose opioid actions (39). In addition, another study shown that pro-inflammatory markers, including IL-1β and TNF-α related to enhancing pain intensity (40). Increased inflammatory variables may also active role in the

progress of many metabolic disturbances, such as type 2 diabetes mellitus or atherosclerosis progression leading to cardiovascular diseases (41)". (line 181-85).

"Also, it was documented that hypovitaminosis D resulted in the dysregulation of insulin sensitivity by reducing adipose PPAR-γ expression and deteriorating β-cell function (14). In addition, gene expression of PPAR-γ was upregulated by vitamin D in tissue of pregnant rats, but its expression was not significantly increased in the liver (45)" (line 194-97).

"The important role of PPAR-γ in regulating motivated behaviors and emotional control is documented with neuroanatomical data showing relatively high concentrations of receptor expression in neurons of the caudate putamen, septum and hypothalamus (48-50)" (line 201-04).

14. English writing needs to be improved.

Authors: Thank you. This point was done and paper edited by a native speaker.

OPEN PEER REVIEW

Revision (2)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (2) 27 Oct 2019

Dear AE,
Comments for the authros:
I read the paper + the revisions however the paper is full of technical problems:
1- Re-arrange your keyword alphabetically.
2- Change the title: The effects of vitamin D supplementation on withdrawal 2 symptoms and gene expression related to inflammatory cytokines and insulin 3 in methadone patients
3- In the method section, this word: This randomized double-blind placebo-controlled clinical trial should be changed into This study
4- In the method section, clearly explain your inclusion and exclusion criteria.
5- Which software and which version was used to calculate the sample size?
6- Which software with which version was used to do the statistical analysis?
7- Reliability and validity of questionnaires should be reported in terms of how you calculated them for your own study.
8- The result section is very short, write more in this section.
9- Add a good and well-organized demographic table to this section. Tables should not be more than 4. Therefore, you can also describe the demographics in one paragraph if adding tables is a problem.
10- Put your tables inside the result section not the end of the paper.
11- Reduce figures to only 4. Put them inside the result section.
12- Limit references to 39 only. 50% of the references should be studied from Iran only.
13- The paper is full of serious grammatical, typo, and editing problems. Ask someone very professional to edit this paper 100% following the end of completing all the revisions. This is a very serious problem in the paper because the English level is really poor.
Best regards

REFEREE: Associate Editor | **Revision** (2) 27 Oct 2019

Dear EIC,
The manuscript is studied, and there are some comments which should be addressed by the author. According to the comments, the document needs major revision.
Kind regards,

REFEREE: EIC | **Revision** (2) 8 Nov 2019

Dear Authors,

Thank you for your recent revisions.

Your paper has now been evaluated again. According to the reviewers' and associate editor's comments it needs some more revisions. Please do your revisions according to these comments. I appreciate you to do the revisions as soon as possible by using the track changes mode in MS Word and using colored text.

Thanks.

OPEN PEER REVIEW

Revision (3)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Associate Editor's Comments:

Associate Editor 1:

Dear correspondence author,

Thank you or sending your revisions to us.

I sent the paper to another professional reviewer and he has asked for very long list of revisions.

Please consider I could not read your paper because it is full of grammatical problems. At this stage, the paper is not good and it should be 100% revised, edited, modified and corrected to be readable please.

Please implement the new revisions in red pen and your previous revisions that were sent to us before, should be in black pen.

Do not make the paper very long, make is well-organised, systematic, well-written and very coherent.

Download our authors` guide for writing original research papers and carefully read it and follow the same.

Regards
Associate editor

Authors: Thank you. First of all, we would like to thank the reviewer for their great comments to improve the quality of the manuscript. We tried our best to revise the manuscript according to the editor's comments.

Reviewer 1:

Dear Sir

I read the paper + the revisions however the paper is full of technical problems:

1- Re-arrange your keyword alphabetically.

Authors: This point was done. This point added to the revised file.

2- Change the title: The effects of vitamin D supplementation on withdrawal symptoms and gene expression related to inflammatory cytokines and insulin in methadone patients.

Authors: Thank you. This point added to the revised file.

3- In method section, this word: This randomized double-blind placebo-controlled clinical trial should be changed into: This study.

Authors: Authors: Thank you. This point added to the revised file.

4- In method section, clearly explain your inclusion and exclusion criteria.

Authors: This point clarified:

“The included subjects were aged 18-60 years, currently undergoing MMT and opioid dependence in the past year. Exclusion criteria were: using colecalciferol 3 months before the intervention and people with history of metabolic diseases, including diabetes, cardiovascular and hypertension before intervention and unwillingness to cooperate during intervention” (Line 78-82).

5- Which software and which version was used to calculate the sample size?

Authors: This point was done. Please see section sample size.

6- Which software with which version was used to do statistical analysis?

Authors: Thank you. This point added to the revised file as follows:

“All statistical analyses conducted using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA)”. Please see section statistical analysis.

7- Reliability and validity of questionnaires should be reported in terms of how you calculated them for your own study.

Authors: Authors: Thank you. This point added to the revised file.

“In the above-mentioned questionnaire, Cronbach’s alpha coefficients ranged between 0.66 and 0.88, while validity and reliability coefficients were 0.73 and 0.88, respectively”.

8- Add a good and well-organised demographic table to this section. Tables should not be more than 4. Therefore, you can also describe the demographics in one paragraph if adding tables is a problem.

Authors: Thank you. This point added to the revised file. Please see Table 2.

9- The result section very short, write more in this section.

Authors: This point was done. Please see revised file.

10- Put your tables inside result section not the end of the paper.

Authors: This point was done. Please see revised file.

11- Reduce figures to only 4. Put them inside the result section.

Authors: This point was done. Please see revised file.

12- Limit references to 39 only. 50% of the references should be studies from Iran only.

Authors: This point was done. Please see references.

13- The paper is full of serious grammatical, typo and editing problems. Ask someone very professional to edit this paper 100% following the end of completing all the revisions. This is a very serious problem in the paper because the English level is really poor.

Authors: Thank you. This point was done and paper edited by a native speaker.

OPEN PEER REVIEW

Revision (3)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Associate Editor | **Revision** (3) 4 Jan 2020

Dear EIC,
The authors have done the revision according to the reviewers' comments.
Thanks.

REFEREE: EIC | **Revision** (3) 18 Jan 2020

Dear Authors,
We have completed our review of your manuscript and are pleased to inform you that it is considered for publication in the IJPBS. Note that the publication of your article will only be finalized upon your cooperation with the editorial processing.
Thank you for your fine contribution. On behalf of the Editors of IJPBS, we look forward to your continued contributions to the Journal.
Thanks.