PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The effect of once weekly folic acid supplementation on erythrocyte folate concentrations in women to determine potential to prevent neural tube defects: A randomised controlled dose-finding trial in Malaysia
AUTHORS	Samson, Kaitlyn; Loh, Su Peng; Khor, Geok Lin; MOHD SHARIFF, ZALILAH; Yelland, Lisa; Leemaqz, Shalem; Makrides, Maria; Hutcheon, Jennifer; Sulistyoningrum, Dian; Yu, Jessica; Roche, Marion; De-Regil, Luz Maria; Green, Tim; Karakochuk, Crystal

VERSION 1 – REVIEW

REVIEWER	Erika Ota St. Luke's International University, Global School of Nursing Science, Global Health Nursing
REVIEW RETURNED	07-Nov-2019

GENERAL COMMENTS	The effect of once weekly folic acid supplementation on erythrocyte folate concentrations in women to determine potential to prevent neural tube defects: A randomized controlled dose-finding trial in Malaysia This paper aims to assess the effect of once weekly folic acid supplementation on erythrocyte folate concentrations in women to determine potential to prevent neural tube defects in Malaysia. Through the author highlights novel findings in this paper, there are some general and methodological issues. I would like to make following comments and suggestions which would improve the quality and readability of this paper. 1 In background, Malaysian situation of folic acid supplementation for NTD should be described, because the research will conduct in Malaysia. 2 In inclusion criteria, please describe women is non-pregnant women. 3. For blinding, please add detail for outcome assessor blinding. 4. In statistical analysis, how do you decide adjustment variables? Please specify. 5. Please consider side effects or adherence should be include in the outcome assessment. 6. Please specify how do you provide supplementation to 2.8mg dose group, do they take supplement once a week? In that case they will know their intervention after intervention start. Other 0.4mg group and placebo group is taking a tablet everyday? Please describe it clearly.

REVIEWER	Rima Obeid Universität des Saarlandes
REVIEW RETURNED	13-Nov-2019

GENERAL COMMENTS	This is a study protocol intends to address an important question of whether the recommendation to supplement high dose folic acid of 2.8 mg once per week is sufficient to increase RBC-folate to the level considered protective against NTDs within an acceptable short time. Specific comments: 1. Abstract: should mention “non-pregnant” women instead of women (line 20). 2. Crider et al, Am J Clin Nutr. 2011 Jun;93(6):1365-72 have published similar study using 4 mg /wk or 0.4 mg/wk. The study mentioned that “The 4000-μg/wk folic acid dose was included in the design of the study because researchers have shown that high-dose folic acid supplements administered weekly can be effective at preventing NTD (e.g. Matern Child Health J 2006;10:397–401). Although the group who received 4000 μg/wk dose (’571 μg/d) consumed a larger total dose than did the group who received 400 μg/d, the former dose was not as effective at lowering high homocysteine concentrations or increasing plasma or RBC folate concentrations as was the 400-μg/d dose. 3. So after reading the study by Crider et al., how to justify the study question and what are the arguments that if the 4 mg was not better than 0.4 mg then the 2.8 mg/wk will be better than 0.4mg? 4. The high dose in some countries aims at improving compliance, not because there is any rational or evidence behind it. 5. The placebo arm is not justified; since we always aim at improving blood folate a minimal dose should be included or the placebo arm should be removed. 6. The MTHFR genotype was the main determinant of the increase in RBC folate and lowering tHcy in response to supplementation (Crider et al. 2011). 7. The sample size estimation can be in theory based on the results of Crider et al. 8. Page 7, Line 49 to 54: folate method should be specified and better described. It is not informative to mention that the method was harmonized. The WHO recommendations of the 906 nmol/L were based on a microbiological assay. 9. What is the method of B12 assay? 10. Why to measure RBP in this study? 11. More thoughts should be given to effect modifiers (the one that are planned and not planned). Examples are MTHFR polymorphism; age, oral contraceptives, BMI, etc. 12. What are the secondary outcomes? Plasma folate will be measured but is seems only for calculating RBC-folate?

VERSION 1 – AUTHOR RESPONSE

REVIEWER 1 COMMENTS:

1. **In background, Malaysian situation of folic acid supplementation for NTD should be described, because the research will conduct in Malaysia.**

In the location section, we note that there is no folic acid fortification program in Malaysia and that folic acid supplementation is not common. Previous studies in Malaysian women of reproductive age have found that B-complex supplements or multivitamin use is low at <10% (Khor *et al*. Asia Pac J Clin Nutr 2006; Green *et al*. Asia Pac J Clin Nutr 2007).[1,2]

Additionally, it should be noted that our study focuses on the WHO guideline for *Intermittent iron and folic acid supplementation in menstruating women*.[3] We have excluded women who are planning on becoming pregnant. Women do not generally take folic acid supplements to prevent NTDs if they are not planning on becoming pregnant. That is why fortification programs were started in the USA and other countries. Daily supplementation with folic acid is not a policy option in women not planning a pregnancy.

2. **In inclusion criteria, please describe women is non-pregnant women.**

This has now been added to the inclusion criteria.

3. **For blinding, please add detail for outcome assessor blinding.**

All laboratory personnel will be blinded to the treatment groups of each participant. Deidentified samples will be sent overseas for analysis, ensuring that the staff analysing the samples will not know which group the participants belong to. As mentioned in the protocol, trial arms will only be unblinded once all data has been collected and entered into the database, and the data analysis plan is finalised.

4. **In statistical analysis, how do you decide adjustment variables? Please specify.**

We have pre-specified adjustment for baseline measures of the outcomes, as these are expected to be strongly related to the outcome. No adjustment for other baseline variables is planned. We have clarified this in the statistical analysis section.

5. **Please consider side effects or adherence should be include in the outcome assessment.**

Adverse events are being recorded, as mentioned in the visit 2 and 3 sections. Adherence is being considered and a secondary 'per-protocol' analysis will also be performed including only women who complete the study and are >80% adherent to the treatment regime.

6. **Please specify how do you provide supplementation to 2.8mg dose group, do they take supplement once a week? In that case they will know their intervention after intervention start. Other 0.4mg group and placebo group is taking a tablet every day? Please describe it clearly.**

There are three treatment arms, all involve women taking a weekly supplement. Thus, blinding is not an issue.

REVIEWER 2 COMMENTS:

1. **Abstract: should mention "non-pregnant" women instead of women (line 20).**

This has now been added to the abstract.

2. **Crider et al, Am J Clin Nutr. 2011 Jun;93(6):1365-72 have published similar study using 4 mg /wk or 0.4 mg/wk. The study mentioned that "The 4000-µg/wk folic acid dose was included in the design of the study because researchers have shown that high-dose folic acid supplements administered weekly can be effective at preventing NTD (e.g. Matern Child Health J 2006;10:397–401). Although the group who received 4000 µg/wk dose ('571 µg/d) consumed a larger total dose than did the group who received 400 µg/d, the former dose was not as effective at lowering high homocysteine concentrations or increasing plasma or RBC folate concentrations as was the 400-µg/d dose.**

   Please see our response below.

3. **So after reading the study by Crider et al., how to justify the study question and what are the arguments that if the 4 mg was not better than 0.4 mg then the 2.8 mg/wk will be better than 0.4mg?**

   We are aware of the study of Crider *et al*.[4] Our study was designed in response to a specific call for research from the WHO in order to provide evidence for the guideline: *Intermittent iron and folic acid supplementation in menstruating women*. Weekly iron and folic acid (IFA) supplementation is recommended in countries where the prevalence of anaemia is above 20% in women of reproductive age as a preventative public health measure. The current WHO recommendation is for 60 mg of elemental iron + 2.8 mg folic acid;[3] thus, this is the dose being tested in our trial.

   Daily folic acid supplementation is more effective at increasing red blood cell folate and would be recommended for a woman planning a pregnancy. However, daily supplementation of folic acid is not a policy option in women not planning a pregnancy. There is a call to remove folic acid from weekly IFA. The only reason to leave it in is that is if a woman was to have an unplanned pregnancy it might prevent her from having an NTD-affected pregnancy. Our research tries to determine which weekly dose is most effective at increasing red blood cell folate.

   The study by Crider *et al*. was based upon the work done by Hao *et al.* 2008.[5] In this study, 400 µg folic acid per day increased red blood cell folate by 300 nmol/L and 4000 µg weekly increased red blood cell folate by 170 nmol/L. Although weekly supplementation was not effective as daily supplementation, this study would seem to support 4000 µg weekly would decrease NTD risk.

4. **The high dose in some countries aims at improving compliance, not because there is any rational or evidence behind it.**

   The reviewer provides no evidence for this practice. Weekly supplementation at higher doses appears to be effective at increasing red cell folate in Crider *et al*. and other papers.

5. **The placebo arm is not justified; since we always aim at improving blood folate a minimal dose should be included or the placebo arm should be removed.**

This study was designed in response to a specific call for research from the WHO and was reviewed by two ethics committees. The study is being done in non-pregnant women of reproductive age, who are not planning on becoming pregnant. If a woman was already pregnant it would be too late to prevent an NTD as the neural tube closes during the first month of pregnancy. If she were to become pregnant, she would not normally be receiving folic acid if not in the study.

Daily folic acid supplementation is more effective at increasing red blood cell folate and would be recommended for a woman planning a pregnancy. However, daily supplementation of folic acid is not a policy option in women not planning a pregnancy. There is a call to remove folic acid from weekly IFA. The only reason to leave it in is that is if a woman was to have an unplanned pregnancy it might prevent her from having an NTD-affected pregnancy.

The WHO has three policy choices:

1. Remove folic acid from weekly IFA
2. Leave it at the current dose used in practice (400 µg/week)
3. Use 2800 µg/week which is the current recommendation but is not widely followed.

Our research tries to determine which weekly dose is most effective at increasing red blood cell folate. It may be that there is no effective dose, thus we must include a placebo.

6. The MTHFR genotype was the main determinant of the increase in RBC folate and lowering tHcy in response to supplementation (Crider et al. 2011).

In the study by Crider *et al.*, the prevalence of MTHFR variant (TT) is incredibly high in this population at 35%. The prevalence worldwide is less than 10%. One of the reasons this study was needed is that this study, in Han Chinese, is not extrapolatable to the world. In the wild type (CC) the % increase in red blood cell folate with daily 4000 µg/week is almost as great as 400 µg/d, 54% versus 64%.[4] Nevertheless, even in the TT variant the weekly dose increased red blood cell folate. It should also be noted that homocysteine is not a relevant biomarker of NTD risk.

7. The sample size estimation can be in theory based on the results of Crider et al.

The sample size in this trial was calculated based on the standard deviation of 202 from Norsworthy *et al.* which examined the effects of the WHO recommended 2.8 mg folic acid weekly on erythrocyte folate concentrations. We increased the sample size from 63 to 100 per group to allow for some uncertainties in the assumed values, particularly the standard deviation. In the study by Hao *et al*. (which is the basis for the study by Crider *et al.*), the average standard deviation across the treatment groups is 242 and the required sample size using this standard deviation is 89 per group. Our sample size of 100 per group therefore remains adequately powered to detect clinically meaningful effects if the standard deviation matches that seen in the study by Hao *et al*.

8. **Page 7, Line 49 to 54: folate method should be specified and better described. It is not informative to mention that the method was harmonized. The WHO recommendations of the 906 nmol/L were based on a microbiological assay.**

The method being used here is the folate microbiological assay. The citation listed in the manuscript is titled: *Harmonizing the Calibrator and Microorganism Used in the Folate Microbiological Assay Increases the Comparability of Serum and Whole-Blood Folate Results in a CDC Round-Robin Study*. The use of the CDC harmonised calibrator and microorganism will promote comparability of folate values amongst laboratories. Nevertheless, the sentence has been revised to say: "De-identified blood samples will be shipped on dry ice to SAHMRI, Adelaide, Australia where plasma folate (nmol/L) and erythrocyte folate (nmol/L) concentrations will be determined using the folate microbiological assay harmonised by the Centers for Disease Control and Prevention".

9. **What is the method of B12 assay?**

B12 will be measured using the Elecsys® 2010 (Roche Diagnostics, Switzerland) automated electrochemiluminescence immunoassay.

10. **Why to measure RBP in this study?**

This has now been removed. The only reason it was mentioned is that RBP comes as part of a group of nutrient biomarkers (along with ferritin, sTfR, CRP, and AGP).

11. **More thoughts should be given to effect modifiers (the one that are planned and not planned). Examples are MTHFR polymorphism; age, oral contraceptives, BMI, etc.**

The purpose of this study is to determine what dose of folic acid, if any, should be included in the weekly IFA. There are no plans for special supplements for the obese and those with the TT variant of the MTHFR. Weekly IFA needs to be a one size fits all approach. As we have mentioned the prevalence of the TT variant is low worldwide. Oral contraceptives in the 1970s have been associated with lower blood folate levels. There is no evidence that modern oral contraceptives affect blood folate indices.[6,7]

We have clarified in the statistical analysis section that there are no planned subgroup analyses.

12. **What are the secondary outcomes? Plasma folate will be measured but is seems only for calculating RBC-folate?**

The secondary outcomes will include plasma folate concentrations at 16 and 20 weeks as well as erythrocyte folate concentrations at 20 weeks across treatment groups. Plasma folate will also be reported as a secondary outcome on its own.

References

1. Geok LK, Duraisamy G, Peng Loh S, Green TJ, Skeaff CM. Dietary and blood folate status of Malaysian women of childbearing age. Asia Pac J Clin Nutr. 2006;15(3):341–9.

2. Green TJ, Skeaff CM, Venn BJ, Rockell JEP, Todd JM, Khor GL, et al. Red cell folate and predicted neural tube defect rate in three Asian cities. Asia Pac J Clin Nutr. 2007;16(2):269–73.
3. World Health Organization. Guideline: Intermittent iron and folic acid supplementation in menstruating women. 2011.
4. Crider KS, Zhu JH, Hao L, Yang QH, Yang TP, Gindler J, et al. MTHFR 677C→T genotype is associated with folate and homocysteine concentrations in a large, populationbased, double-blind trial of folic acid supplementation. Am J Clin Nutr. 2011;93(6):1365– 72.
5. Hao L, Yang QH, Li Z, Bailey LB, Zhu JH, Hu DJ, et al. Folate status and homocysteine response to folic acid doses and withdrawal among young Chinese women in a large-scale randomized double-blind trial. Am J Clin Nutr. 2008;88(2):448–57.
6. Green TJ, Houghton LA, Donovon U, Gibson RS, O'Connor DL. Oral contraceptives did not affect biochemical folate indexes and homocysteine concentrations in adolescent females. J Am Diet Assoc. 1998;98(1):49–55.
7. Wilson SMC, Bivins BN, Russell KA, Bailey LB. Oral contraceptive use: Impact on folate, vitamin B6, and vitamin B12 status. Nutr Rev. 2011;69(10):572–83.

VERSION 2 – REVIEW

REVIEWER	Erika Ota St. Luke's International University, Global School of Nursing Science, Global Health Nursing
REVIEW RETURNED	26-Dec-2019

GENERAL COMMENTS	Thank you very much for the revision, no more comments.