PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

Title (Provisional)

Serum folate and dietary folate intake in beta thalassaemia trait: A case control study from Sri Lanka

Authors

Thilakarathne, Shyamali; Jayaweera, Udayanga Prasad; Herath, Thushari Uthpala; Silva, Renuka; Premawardhena, Anuja

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VERSION 1 - REVIEW

Reviewer	**1**
Name	**Adam, Ishag**
Affiliation	**University of Khartoum, Faculty of Medicine**
Date	**21-May-2024**
COI	**None**

The sample size is not calculated perfectly

I suggest to recalculate it using the difference in the mean (for the folate level) and proportion (food intake).

Statistics

looking for confounders through multivariate analysis is necessary.

Reviewer	**2**
Name	**Chakrabarti, Prantar**
Affiliation	**Zoho Corporation, Hematology**
Date	**10-Jul-2024**
COI	**None, except that I have met Prof. Anuja in some conferences.**

An excellent, timely study. I would think if the folate levels between the two groups were depicted visually using bar charts or any other similar graphical description, it would be easier for the readers to visualize that there is no significant difference between the two groups

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Reviewer	**3**
Name	**Darbandi, Bahram**
Affiliation	**Pediatric Growth Disorders Research Center**
Date	**18-Oct-2024**
COI	**I agree BMJ's co-reviewing policy and peer review terms and conditions**

I don't have any comments to the auther

Reviewer	**4**
Name	**Katewa, Vikash**
Affiliation	**SN Medical College**
Date	**07-Nov-2024**
COI	**None**

Following issues need to be addressed while revising

1. In the result section of abstract (Page 4) , The results suggested 34% of cases (mean; 4.88 ng/mL) and 24% of controls (mean;4.76 ng/mL) had serum folate deficiency (<3 ng/mL), here the statement creates confusion to readers as the value of Serum folate has been shown < 3 ng /ml while the mean value has been mentioned as 4.88 and 4.76 ng/ml (this mean represent for whole cohort , so it should be either presented as separate sentence rather than mixing with the deficient percentage.

2. Author has not mentioned from where the thalassemic trait patients were recruited (whether there is any registry for Thal trait or a large number of children were screened by HPLC to get a figure of 100 thal trait children , if it is so what was the indication for doing HPLC in these children/patients).

3. In method section the minimum age mentioned as 5 years while in tables the minimum age group is mentioned from 6 years onward

4. On page no 9 : author has mentioned "Out of 100 pairs, 78

(39%) were from urban areas, while 61 (61%) were dwellers from rural areas, the number 78 should be 39 as the description here is for pairs rather then for the total numbers.

5 As per the methodology the numbers of control were 100 but on page number 10, the prevalence of underweight has been calculated for 98 controls, similarly in the table 2 , for the comparison the number of cases is mentioned as 94 while control as 91(lack of consistency in data) . Authors has no where mentioned why this difference in data has occurred

6. Usually beta thalassemia trait are asymptomatic except mild anemia and there is no specific dietary advise is given to them , than it is obvious that in same household there will be no difference in dietary intake of folate and protein , so why this matching was done in same household to compare the dietary protein and folate intake , it does not serve any benefit

7.How the groups were divided based on income (Is the given range are arbitrary)

8. In the discussion section why the author has focused on risk of folate supplementation (Reference 28 to 36) as it was neither the objective of study nor this parameter was studied by author, so why the irrelevant and lengthy discussion on risk of folate supplementation has been mentioned.

9.On page number 8 author has classified thalassemia as heterzygous beta thal based on Hb A2 report rather it should be mentioned as Thalassemia trait as zygosity is based on genetic report

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VERSION 1 - AUTHOR RESPONSE

Reviewer 1	
1. The sample size is not calculated perfectly I suggest to recalculate it using the difference in the mean (for the folate level) and proportion (food intake).	Justification: • Sample size was calculated to detect the mean difference in folate levels between the BTT and normal adults. We used following assumptions; o Mean difference = 6 (ng/mL) o Standard deviation = 15 (ng/mL) o Significance level = 5% o Power 80% o Equal number of cases and controls

	• Accordingly, sample size was calculated as 98, therefore, we decided to recruit 100 for cases and controls. • We have already completed the study and recalculation of sample size is not feasible. We realized that the sample size calculation has not been fully explained in the manuscript and we corrected it. Methodology WinPepi software, version 11.65, was used to calculate sample size at the significance level of 5%, power 80%, mean difference of 6 ng/mL and standard deviation of 15 ng/mL for equal number of cases and controls.[13,14]
2. looking for confounders through multivariate analysis is necessary.	Results In the stage of study designing (sampling) demographic/socioeconomic characteristics and anthropometric measurements which would be confounders were controlled by matching cases and controls with nearly similar characteristics. Therefore, the effect of confounders due to the above variables on the final outcomes is likely to be minimum. Nevertheless, respecting the wishes of the reviewer, we conducted a regression analysis. However, none of the variables (age, sex, ethnicity, sector, level of income, height & weight) was identified as a confounder for serum folate level and dietary folate level.
Reviewer 2	
1. An excellent, timely study. I would think if the folate levels between the two groups were depicted visually using bar charts or any other similar graphical description, it would be easier for the readers to visualize that there is no significant difference between the two groups	We respect the request of reviewer 2 and have included a bar diagram. However, we must add that it leads to the repetition of what we say in the text.

Reviewer 3 (no comments)	
Reviewer 4	
1. In the result section of abstract (Page 4) , The results suggested 34% of cases (mean; 4.88 ng/mL) and 24% of controls (mean;4.76 ng/mL) had serum folate deficiency (<3 ng/mL), here the statement creates confusion to readers as the value of Serum folate has been shown < 3 ng /ml while the mean value has been mentioned as 4.88 and 4.76 ng/ml (this mean represent for whole cohort , so it should be either presented as separate sentence rather than mixing with the deficient percentage.	Abstract: The results suggested 34% of cases and 24% of controls had serum folate deficiency (<3 ng/mL), while 37% of cases and 49% of controls were at risk (3-5.9 ng/mL) for folate deficiency. Overall, the serum folate level was not significantly different between the cases (mean; 4.88 ng/mL) and the controls (mean; 4.76 ng/mL) (p=0.759). Dietary folate intake was low (<RDA) in both groups but not significantly different between the groups.
2. Author has not mentioned from where the thalassemic trait patients were recruited (whether there is any registry for Thal trait or a large number of children were screened by HPLC to get a figure of 100 thal trait children , if it is so what was the indication for doing HPLC in these children/patients).	Already addressed the comment; Please see the response to editors comment No 4.
3. In method section the minimum age mentioned as 5 years while in tables the minimum age group is mentioned from 6 years onward	Methodology; This study was a case-control study including 100 sets of samples aged between 6-25 years.
4. On page no 9 : author has mentioned "Out of 100 pairs, 78 (39%) were from urban areas, while 61 (61%) were dwellers from rural areas, the number 78 should be 39 as the description here is for pairs rather then for the total numbers.	Results; Out of 100 pairs, 39 (39%) were from urban areas, while 61 (61%) were from rural areas.
5. As per the methodology the numbers of control were 100 but on page number 10, the prevalence of underweight has been calculated for 98 controls, similarly in the table 2 , for the comparison the number of cases is mentioned as 94 while control as 91(lack of consistency in data) . Authors has no where mentioned why this difference in data has occurred	Methodology; However, when determining the full blood count parameters, complete information could be obtained from 94 cases and 91 controls only. Similarly, prevalence of anaemia and underweight among control group were determined using data from 98 participants due to the missing of data.
6. Usually beta thalassemia trait are asymptomatic except mild anemia and there is no specific dietary advise is given to them , than it is obvious that in	Justification; Beta thalassaemia trait is associated with increased ineffective erythropoiesis (IE); though

	same household there will be no difference in dietary intake of folate and protein , so why this matching was done in same household to compare the dietary protein and folate intake , it does not serve any benefit	it's not "clinically significant" to cause significant symptoms in the most. With IE, there will be an increase in the demand for folate to match the red cell turn over. If this happens in a back drop of dietary folate deficiency it can exacerbate folate use and have a deleterious effect for red cell production. The matching from the same household was done to negate the effect of dietary folate intake which can happen due to socio economic variabilities.
7.	How the groups were divided based on income (Is the given range are arbitrary)	Justification; The information on income level was obtained using an open-ended question. To make the statistical analysis easier, income levels were divided in to groups. Care was taken to interpret income levels based on 'Household Income and Expenditure Survey 2019- Sri Lanka'.
8.	In the discussion section why the author has focused on risk of folate supplementation (Reference 28 to 36) as it was neither the objective of study nor this parameter was studied by author, so why the irrelevant and lengthy discussion on risk of folate supplementation has been mentioned.	Justification; We value the reviewers' comment on the superfluous nature of discussing the risk of folate supplementation. However, what we wanted to highlight is that indiscriminate supplementation of folic acid for beta thalassaemia trait is not needed and could even be harmful.
9.	On page number 8 author has classified thalassemia as heterzygous beta thal based on Hb A2 report rather it should be mentioned as Thalassemia trait as zygosity is based on genetic report	Replaced with "beta thalassaemia trait"

VERSION 2 - REVIEW

Reviewer	**4**
Name	**Katewa, Vikash**
Affiliation	**SN Medical College**
Date	**31-Dec-2024**
COI	

The revised manuscript is appropriate and authors has included the suggested changes in the revision. The discussion part containing the folate supplementation and associated risk factor still seems lengthy and inappropriate as authors have not studied this part and would have better if this was shortened

VERSION 2 - AUTHOR RESPONSE

Reviewer 4	
1. The revised manuscript is appropriate and authors has included the suggested changes in the revision. The discussion part containing the folate supplementation and associated risk factor still seems lengthy and inappropriate as authors have not studied this part and would have better if this was shortened	Discussion As suggested, the discussion part containing the folate supplementation and associated risk factors was shortened. Some of the references were removed as well.