Author’s response to reviews

Title: Shine & Lal index as a predictor for early detection of β-thalassemia carriers in a limited resource area in Bandung, Indonesia

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Version: 2 Date: 10 Jun 2019

Author’s response to reviews:

Dear Editor
BMC Medical Genetics

Thank you for your valuable comments for our manuscript "Shine & Lal Index as a predictor value for early detection of β-thalassemia carriers in a limited source area in Bandung, Indonesia" (MGTC-D-18-00381R1).

Please find below some essential revisions suggested by your reviewers in a point-by-point response, written in blue letters.

Your reviewer has commented about the use of English in our manuscript. We apologize for this.

Maria de Fatima Sonati (Reviewer 1):
The manuscript is better now, but still needs corrections.
Firstly, a review of English.
Thank you again for your suggestion.
We have consulted for the English, however, some editors refused with reason due to deep content of the subject. We have re-written the story line of this manuscript. If this new version still does not meet the requirement, please advise whether we need to involve the English Service from the BMC, therefore, we are willing to pay for the service.

Thank you
Furthermore, we would like to change the title by deleting the word value if it meets the requirement.

"Shine & Lal Index as a predictor value for early detection of β-thalassemia carriers in a limited source area in Bandung, Indonesia"

Thank you

Technical Comments:

1. Please change the 'Materials and Methods' section header to 'Methods'.
   Thank you for your correction.
   We have changed the section header to “Methods” as you requested.

2. In both Methods and Declarations sections, please declare whether written or verbal informed consent was obtained from all participants. If verbal, please state the reason and whether the ethics committee approved this procedure.
   Written informed consent was obtained from all participants.
   Please find the revised statement in section Methods and section Declaration:

Participants and sample collection
First degree of family members, including father and/or mother and/or siblings were invited to participate in the screening of thalassemia carrier status during a family gathering of thalassemia patients in Dr. Hasan Sadikin General Hospital, Bandung, Indonesia. In this family gathering, a mini seminar was held, involving professional speakers including Pediatrician, Clinical Pathologist, and the Indonesian Thalassemia Foundation. Written consent was obtained from all adult participants. A letter form of carrier screening agreement for children younger than 16 years old was signed by their parents.

DECLARATION
Ethics approval and consent to participate
The ethical clearance was granted by the Ethics Committee of Faculty of Medicine, Universitas Padjadjaran (http://kepk.fk.unpad.ac.id/) no. 966/UN6.C.10/PN/2017
Written consent was obtained from all adult participants. A letter form of carrier screening agreement for children younger than 16 years old was signed by their parents.

3. Please remove the Ethics approval document and the consent form from the Declarations and the supplementary files.
   Thank you for your suggestion.
   We have removed the Ethics approval document and the consent form from the Declarations and the supplementary files as you requested.

4. Consent for publication refers to consent for the publication of identifying images or other personal
or clinical details of participants that compromise anonymity. Seeing as this is not applicable to your manuscript please state “Not Applicable” in this section.
Thank you for your suggestion.
We have removed the previous statement and have changed to the statement into “Not applicable”

5. In the 'Funding' statement, please declare the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.
Thank you for your suggestion.
We have written as follows:

Funding
This study is funded by The Academic Leadership Grant (ALG) Universitas Padjadjaran 2017, given to DSH as principal investigator.
Universitas Padjadjaran as the funding body has no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

6. Please remove the funding information from the Acknowledgements and include it in the Funding section instead.
Thank you for your suggestion.
We have removed the funding information from the Acknowledgements and include it in the Funding section instead:

Funding
This study is funded by The Academic Leadership Grant (ALG) Universitas Padjadjaran 2017, given to DSH as principal investigator.
The funding body Universitas Padjadjaran has no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Acknowledgement
We are grateful to the Indonesian Thalassemia Foundation (YTI) and the Indonesian Association for Parents of Thalassemia Patients (POPTI) in Bandung, West Java for organizing the family gathering event.

7. Please provide figure titles/legends under a separate heading of 'Figure Legends' after the References. Figure files should contain only the image/graphic, as well as any associated keys/annotations. If titles/legends are present within the figure files, please remove them.
Thank you for your suggestion.
We have put the figure titles/legends under a separate heading after the References. Hope this meets your requirement

Figures should be provided as separate files, and each figure of a manuscript should be submitted as a single file.
Thank you for your suggestion.
We have submitted each figure as a single file.
In total there are 2 figures and 1 table, thus, there are 3 separate files

Please ensure that all figures/tables and supplementary files are cited within the text. Any items which
are not cited may be deleted by our production department upon publication.
Thank you for your note
We have checked all figures/tables and supplementary files.

Reviewer reports:

Maria de Fatima Sonati (Reviewer 1):
The manuscript is better now, but still needs corrections.
Firstly, a review of English.
Thank you again for your suggestion.
We have consulted for the English, however, some editors refused with reason due to deep content of the subject. We have re-written the story line of this manuscript. If this new version still does not meet the requirement, please advise whether we need to involve the English Service from the BMC, therefore, we are willing to pay for the service.
Thank you

In the first lines of the Abstract and Introduction, the thalassemia concept must be adequate: globins do not mutate, globin-genes mutate.
Thank you for your comment.

Abstracts
Background. Thalassemia is the most common inherited disease in the world, involving α- or β-globin of red blood cell.

Background
Thalassemia is an inherited disease, caused by mutations in α- or β-globin gene.

What is the value of MCH used for cutting, 26 pg, as stated in the Abstract, or 27 pg, as stated in the Methods?
Thank you for your correction
We apologize to confuse with different number.
The MCH value used as cut off is 27 pg, and we have corrected throughout the manuscript.

Abstract
Those with MCV <80fL and/or MCH <27pg and/or SLI <1530 were further examined for Hb analysis

Methods
Only those who had MCV <80fL and/or MCH <27pg and/or Shine & Lal index <1530 were further subjected for Hb analysis using capillary electrophoresis (Minicap Sebia, France).

Hb E is a variant, but Hbs A, A2 and F are normal hemoglobin, can not be so termed. They are fractions of a normal hemolysate. Please correct, in Abstract, in the Methods and where else is so referred to.
Thank you for your suggestion
We have re-checked and re-written the terminology, hope it suits the definition as suggested.

In the Abstract
(Results, line 14) it would be more appropriate to say Hb E only, not 'high Hb E level';
Thank you for your suggestion.
We have removed the words high and level
Only samples with HbA2 fraction >4% or with a peak of HbE fraction were sequenced to confirm β-globin gene mutations. Results. Of 196 family members, 117 (59.6%) had low MCV and/or low MCH and/or low SLI. HbE fraction (mean 24.06% ± 0.95, range 22.4-26.5) was found in 27 (13.7%) cases, all had a mutation at codon (CD)26 (c.79G>A) and the mean of HbA2 fraction in these samples was 3.18%+0.62 (range 2.6-3.8).

in line 15, replace 'genetic test' by molecular analyzes or DNA sequencing or beta-globin gene sequencing.
Thank you for your suggestion.
We have re-written the phrase;

Previous abstract:
Results. A total of 117 of 196 (59.6%) family members had low MCV and/or MCH and/or SLI, of whom high HbE level (mean 24.06% ± 0.95) was found in 27 (13.7%) cases, and all confirmed by genetic test, revealing mutation at codon (CD)-26 (c.79G>A).

Current abstract:
Results. Of 196 family members, 117 (59.6%) had low MCV and/or low MCH and/or low SLI. HbE fraction (mean 24.06% ± 0.95, range 22.4-26.5) was found in 27 (13.7%) cases, all had a mutation at codon (CD)26 (c.79G>A) and the mean of HbA2 fraction in these samples was 3.18%+0.62 (range 2.6-3.8).

At line 16, the number and percentage of samples with Hb A2 above 4% should be informed.
Thank you for your suggestion.

Previous abstract:
“…..Samples with HbA2 >4% were all confirmed as β-thalassemia carrier who had mutation, mostly at c.92+5 G>C (IVS1nt5; n28), c.27_28insG (CD-8/9; n1) and c.59A>G (CD-19; n1)…..”

Current abstract:
“…..For samples with HbA2 >4% (n30; 15.3%), all had mutation at IVS1nt5 (c.92+5 G>C; n28), CD8/9 (c.27_28insG; n1) and CD19 (c.59A>G; n1). The mean HbA2 fraction with mutation at IVS1nt5 (c.92+5 G>C) was 4.65%+0.77 (range 4.0-5.6)…..”

At the end of the Introduction
(page 2, line 7), 'Since more than 200 different THALASSEMIC mutations' have been ...
Thank you for your correction
We have put the word thalassemic as you suggested

Moreover, since there are more than 200 different thalassemic mutations have been reported in the β-globin gene, it becomes necessary to confirm the diagnosis and to map the mutation spectrum among population.

In Methods,
'Ethical Approval' must come before, or immediately after or in 'Participants'.
Thank you for your input.
We have reordered the ethical approval at the end of participants and sample selection section.
Participants and sample collection
First degree of family members, including father and/or mother and/or siblings were invited to participate in the screening of thalassemia carrier status during a family gathering of thalassemia patients in Dr. Hasan Sadikin General Hospital, Bandung, Indonesia. In this family gathering, a mini seminar was held, involving professional speakers including Pediatrician, Clinical Pathologist, and the Indonesian Thalassemia Foundation. Written consent was obtained from all adult participants. A letter form of carrier screening agreement for children younger than 16 years old was signed by their parents. Venous blood was collected ……..
The protocol of the study was approved by the Ethics Committee of Faculty of Medicine, Universitas Padjadjaran, with ethical clearance no. 966/UN6.C.10/PN/2017

Detection of mutation in the beta-globin GENES rather than Beta-globin mutation detection (Methods). Thank you for your correction
We have re-written the sub title into Detection of mutation in the β-globin GENES as suggested, but we deleted the S from GENES, because it is only one β-globin Gene

Detection of mutation in the β-globin gene
DNA was isolated from EDTA venous blood…….

In the penultimate paragraph of the Discussion, it would be important to justify the statement included and explain the laboratory consequences of co-existence of alpha and beta thalassemias.
Thank you for your suggestion.
We put this statement in the limitation of the study.

This study had several limitations; lack of family pedigree to establish genotype/phenotype segregation is fairly major issue in this study. Better data base of family members’ registry is recently being set up. Another limitation in this study is that iron profile examination is lacking. Furthermore, respondents with low Shine & Lal index who have normal or borderline HbA2 are not further examined for the possible other α-globin gene mutations, therefore, DNA analysis need to be performed since co-inheritance of IDA and β-thalassemia, and also of α- and β-thalassemia might occur even in rare cases.24,25

Reviewer 2 (Reviewer 2): PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses one or several testable research questions? (Brief or other article types: is there a clear objective?)
Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?
Yes - the approach is appropriate
EXECUTION - Are the experiments and analyses performed with sufficient technical rigor to allow confidence in the results?
Yes - experiments and analyses were performed appropriately

STATISTICS - Is the use of statistics in the manuscript appropriate?
No - there are issues with the statistics in the study
Thank you for your comment.
We have re-written as follows:

Statistical analyses
Anemia prevalence was compared between groups of common β-globin mutations (c.79G>A vs. c92+5G>C) using χ-square test. The value of Hb fractions (HbA2, HbA and Hb E) was checked whether the data was normally distributed, and the mean values were compared between groups of common β-globin mutations using t-test. Analysis was performed by SPSS v.22 for Window, licensed for Universitas Padjadjaran.

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?
Yes - the author's interpretation is reasonable

OVERALL MANUSCRIPT POTENTIAL - Has the author addressed your concerns sufficiently for you to now recommend the work as a technically sound contribution? If not, can further revisions be made to make the work technically sound?
Probably - with minor revisions

PEER REVIEWER COMMENTS:

GENERAL COMMENTS:
The authors have made a good effort to address all of the points made in the initial review and have made some substantial amendments to the manuscript. This is now a much improved manuscript and makes a much better case for the study data and aims.

REQUESTED REVISIONS:
There are still spelling and grammatical inaccuracies throughout the manuscript; maybe some editorial input could be sought to remedy this.
Thank you for your suggestion.
We have consulted an editor, however, may be because she is not really into the topic, the manuscript is still lacking spelling and grammatical inaccuracies.
Please advise what to do to make our manuscript in a better English.
We are willing to use your English Service from BMC and pay for it.

Another issue is that the Statistics section of the Methods is too scant: details of the statistical platform used (even if Excel) should be given, along with the method of establishing normality.
Thank you for your comment.
We have used SPSS instead to calculate and analyse.
We have stated the SPSS in the manuscript now.

Statistical analyses
Anemia prevalence was compared between groups of common β-globin mutations (c.79G>A vs. c92+5G>C) using χ-square test. The value of Hb fractions (HbA2, HbA and Hb E) was checked whether the data was normally distributed, and the mean values were compared between groups of common β-globin mutations using t-test. Analysis was performed by SPSS v.22 for Window, licensed for Universitas Padjadjaran.

ADDITIONAL REQUESTS/SUGGESTIONS:
None in addition to above.