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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Author’s response to reviews:

Bandung 28 April 2019

Dear Editor,

We would like to resubmit our study in your journal entitled

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

Thalassemia ranks 5th in the list of national catastrophic diseases in Indonesia, however, nationwide screening for thalassemia carrier is not yet mandatory. Our previous study showed that the common mutations in β-thalassemia carrier in West Java are predominantly at codon-26 (c.79G>A) and at IVS1nt5 (c.92+5 G>C).

In a limited source area such as Indonesia where HbA2 examination and DNA analysis are scarce, we are struggling to do campaign for early detection for thalassemia carrier. Our study has shown that a simple blood examination using hematological indices including MCV, MCH and Shine&Lal index (MCV*MCV*MCH/100) play an important role in early detection of thalassemia carriers.

We hope this study may encourage regions, where molecular method is not readily available, to early detect β-thalassemia by using simple complete blood count indices such as MCV, MCH, the Shine & Lal index.
Please find below the detailed point-by-point response to the reviewers’ comments and the manuscript with ‘comment mode on’ with the correction. Thank you and hoping to have a good review from you

Best regards

Ani Melani Maskoen

Detailed point-by-point response to the reviewers’ comments

Thank you all comments from the reviewers. We also have attached the manuscript with your comments and our reply in the supplementary section.

Reviewer reports:
Maria de Fatima Sonati (Reviewer 1):
The manuscript is well written, of practical interest and technically sound. We have only three suggestions to make to the authors:
1. statistical analyzes should be repeated using non-parametric test, since not often the hematimetric values do not present normal distribution;
   Thank you for your comment. In our study, the value is in a normal distribution
2. the penultimate paragraph of the Discussion should explore and better explain to the reader the effects of the limitations indicated by the authors, particularly the lack of investigation of the status of alpha genes;
   Thank you for your comment. Please find attached the new version for the Discussion section
3. Figure 1 is unnecessary.
   Thank you for your suggestion, we have removed the figure.

Reviewer 2 (Reviewer 2): PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses a testable research question(s) (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?
No - there are minor issues

EXECUTION - Are the experiments and analyses performed with technical rigor to allow confidence in the results?
No - there are minor issues

Statistics - Is the use of statistics in the manuscript appropriate?
No - there are issues with the statistics in the study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?
No - there are minor issues
OVERALL MANUSCRIPT POTENTIAL - Is the current version of this work technically sound? If not, can revisions be made to make the work technically sound?
Maybe - with major revisions

PEER REVIEWER COMMENTS:

GENERAL COMMENTS: This is a well conceived, but poorly executed study, aiming to investigate the utility of several full blood count metrics in the screening of beta-thalassemia carriers, in a region where molecular methods are not readily available. Unfortunately, due to logistical and financial limitations (recognised and discussed in the manuscript) the data was not conclusive. Also, the use of language and non-standard gene nomenclature further hamper what could otherwise be an interesting (if minor) addition to the field.
Dear Reviewer,
We appreciate your comments.
Please find below our answers to your comments in capital letters.
We apologize to use capital letters to be able to distinguish between the ‘comments section’ and the ‘answer section’.

Thank you for your understanding.

REQUESTED REVISIONS:

General comments:
HGNS nomenclature must be used for all gene names and HGVS nomenclature should be followed for the description of all variants; the legacy nomenclature currently used can be added in parenthesis on first use, e.g.: HBB c.93+1G>T (IVS1nt1). The transcript accession number used for the analysis should also be given on first use, e.g., for HBB, NM_000518 should be given if used.
Syntax, grammar and other typography are not to required standard; must recruit editorial input from someone necessarily proficient in English.

Specific comments:

Page2Line8:
is 'thalassemia' a single gene disorder? This could cover both a- and b- thalasemia, which affects two gene loci;
THANK YOU FOR YOUR CORRECTION.
INDEED THALASSEMIA CAN BE CATAGORIZED AS a-THAL AND b-THAL. WE CHANGE THE SENTENCE INTO: THALASSEMIA IS THE MOST COMMON INHERITED DISEASE. WE ALSO CHANGE THE POSITION OF THALASSEMIA IN THE NATIONAL CATASTROPHIC DISEASES IN INDONESIA SINCE THE NEW UPDATE OF THALASSEMIA IS INCREASED TO 5TH please specify b-thalassaemia in this context and elsewhere in the text).
THANK YOU FOR YOUR COMMENT.
WE PUT β-THALASSEMIA THROUGHOUT THE MANUSCRIPT TO BE MORE SPECIFIC.

P2L15:
should be: 'not yet mandatory'. Also, is mandatory the word the authors are looking for here? TERM MANDATORY TO INDICATE THAT THE SCREENING IS NOT A MUST. WE CHANGE TO NOT YET MANDATORY.

P3L19: there should be discussion about the genetics of b-thal, inheritance patterns and difference between (in terms of clinical severity) of the carrier (trait) and disease (biallelic) states. THANK YOU FOR YOUR INPUT. WE HAVE RE-WRITEN THIS IN THE FIRST PARAGRAPH, AND WE HAVE CHANGED THE REFERENCES IN THE GOOD ORDER.

P3L29: mean what? (MCH); Mean Corpuscular Hemoglobin also, laboratory, not laboratorium. THANK YOU FOR YOUR CORRECTION

Also, there should be a description of how the various blood count metrics relate to b-thalassemia THANK YOU, WE HAVE PUT PARAGRAPHT STATING THIS. and how these are used for a differential diagnosis. THANK YOU, WE HAVE PUT PARAGRAPHT STATING THIS

Simply linking to references is not sufficient - do not assume expert-levels of knowledge in the prospective readership. THANK YOU, WE HAVE PUT THE INFORMATION HEREABOUT STATING THIS.

P3L41: haemoglobin E, not haemoglobinopathy E? THANK YOU FOR YOUR CORRECTION

P4L22: need to introduce the concept of HBB pathogenic variants, gene structure and association with disease phenotype before discussing actual variants. These should also be defined according to HGVS nomenclature. THANK YOU FOR YOUR COMMENT. THIS IS STATED IN INTRODUCTION NOW.

P4L36: HBB gene; also, do the primers used span the whole gene (including 5' and 3' UTRs and introns)? Unless commercially sensitive, the primer sequences should be given (along with any references relating to design & validation). THANK YOU FOR YOUR INPUT. WE HAVE PUT THE PRIMER IN THE TEXT

P5L39: there is no Figure 4… THANK YOU FOR YOUR COMMENT. WE HAVE REMOVED THE TEXT.
P6L21:
57.1% is not a small percentage!! 25.9% is not a 'small' percentage either… this line of argument must be reframed to reflect the data.
THANK YOU FOR YOUR SUGGESTION, WE HAVE CHANGED THE STATEMENT

P6L51:
'some machines'…? Do the authors mean capillary electrophoreses platforms? Please use precise technical language.
THANK YOU FOR YOUR SUGGESTION.

P7L16:
first mention of a-thalassemia, this and similarities and differences with b-thal - should have been introduced in the Introduction.
THANK YOU FOR YOUR COMMENT. WE HAVE PUT ABOUT aTHAL IN THE INTRODUCTION

P7L29:
what do the authors mean by 'towards zero birth of thalassemia'? The authors should think very carefully about the implications of this statement and either clarify or remove.
THANK YOU FOR YOUR SUGGESTION. DURING A THALASSEMIA SEMINAR SOME TIME AGO, THE GOAL IS INDEED THE ZERO BIRTH OF THALASSEMIA IN THE YEAR 2030. AFTER SOME DISCUSSION, WE THINK IT IS BETTER TO REMOVE THIS STATEMENT.

P7L31:
the lack of pedigree data to establish genotype/phenotype segregation is a fairly major issue with this study;
INDEED, HOWEVER, SOME OF THEM ALSO HESITATE TO WRITE UP THE PEDIGREE, THINGS THAT WE COULD NOT FORCE

citing 'lack of time' is not acceptable for a scientific study.
THANK YOU, WE REMOVE THE STATEMENT

Attached in the supplementary file:
Manuscript with comments from the reviewers and our reply