Author’s response to reviews

Title: Exon sequencing of the alpha-2-globin gene for the differential diagnosis of central cyanosis in newborns: a case report

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We would like to thank the editor and reviewers of the BMC pediatrics for taking the time to review our article. We have made some corrections and clarifications in the manuscript after going over the reviewers’ comments. The changes are summarized below.

Reviewer reports:

Maria de Fatima Sonati (Reviewer 1):

1. In the title and in the abstract, one could use alpha-2-globin gene instead of 'hemoglobin gene'
   = We have changed “hemoglobin gene” to “alpha-2-globin gene”.

2. Regarding the mutation detected, if paternity tests were not performed, it would be better to refer to it as a 'probable' de novo mutation.
   = Unlike the newborn infant, the genetic evaluation of HBA2 in his parents was normal for the mutation. This appears to be a de novo mutation.
Nasir Al-Allawi, MBChB, PhD, FRCPath (Reviewer 2):

There are few issues that may be worth considering:

1. Abstract: in the conclusions page 2 line 51-52. The statement needs to be modified. And it could be said that methemoglobinemia maybe fatal in severe cases, not generally fatal as in the statement.

   = We have changed the paragraph as described below.

   Hb M disease is a benign disease and does not require any treatment whereas acquired methemoglobinemia is a potentially fatal condition.

2. Background page 3 lines 9-12, the statement should be clarified. It could be mentioned that Hb M is one of the causes of inherited Methemoglobinemia. Or they could mention the enzyme deficiencies as well as Hb M are a cause of inherited Methemoglobinemia.

   = The paragraph has been corrected.

   Hemoglobin M (Hb M) is one of the causes of inherited methemoglobinemia.

3. Page 4 lines 9-12, What method was used to determine the Hb variants, was it HPLC, cellulose acetate, or capillary electrophoresis?

   = We have changed the paragraph as described below.

   Hemoglobin electrophoresis at alkaline pH on agarose gel showed normal age profiles with 82.7% Hb F, 17.2% Hb A1, and 0% Hb S.

4. Page 4 lines 14-19, Actually to my knowledge HBA2 c.175C>T which leads to Hb M Boston involves Amino Acid 58 as per references cited by the authors. Could the authors double check this and correct if necessary.

   = The Globin Gene Server (https://lovd.bx.psu.edu/) for alpha-2 globin and the ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) database at the National Center for Biotechnology Information (NCBI) demonstrate that Hb M Boston could have the protein change of “p.His58Tyr” or “p.His59Tyr”.

   = We have changed the paragraph as described below.

   Hemoglobin electrophoresis at alkaline pH on agarose gel showed normal age profiles with 82.7% Hb F, 17.2% Hb A1, and 0% Hb S.
Reviewer 2 (Reviewer 3)

* Recommend authors to explain in more details what makes this case different from previously reported cases.

As described in the second paragraph at discussion section, we presented the case with hemoglobin M variant due to a de novo mutation at the α2-globin. Although de novo mutations are not rare events in human genetic diseases, relatively few have been recorded for the occurrence of the mutation in patients with hemoglobin M. Especially, it is rarer at the cases with α-chain mutants than β-chain mutants. It can also be seen in the Table 1 (added in the revised version of manuscript). We would like to highlight that Hb M should be considered in the differential diagnosis of cyanosis in the newborn period, even if no familial cases are detected.

* If authors want to review available literature, I recommend summarizing previously reported cases in a table including important information such as year of publication, disease presentation, diagnosis, etc.

We have added the table as described below.

Table 1 Summary of hemoglobin M diseases presenting as neonatal cyanosis

* In the method section, please clarify what diagnostic tests were performed to rule out cardiac and pulmonary disorders.

We have changed the paragraph as described below.

After ruling out respiratory and cardiac causes based on chest radiographic and echocardiographic studies, further evaluation for hemoglobin derivatives incapable of binding oxygen was conducted.

Also, we have corrected the formatting of Declarations section requested from Editorial Office.

We hope the revised manuscript will better meet the requirements of your journal for publication. We thank the editor and the reviewers of the BMC pediatrics once again for the constructive review of our paper.

Sincerely yours,

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