Author's response to reviews

Title: Echocardiographic Evaluation of Thalassemia Intermedia Patients in Duhok, Iraq

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Author's response to reviews: see over
The author's response to reviews

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The author's response:

The author highly appreciates the reviewers’ constructive comments. He believes his revision of the manuscript contributes to improve its quality and presentation. A detailed point-by-point response to each of the reviewers’ queries is attached.
July 10th, 2014

To editors,
BMC Cardiovascular Disorders. (Non-coronary section)

Dear Mr Gilbert Tacbobo,

I would like to thank you for revising the paper. The reviewers’ comments have been addressed properly in the revised version.

Reviewer 1: Mehran Karimi

A) How the sample size is calculated?

Author response: The point is added as required. The total number of TI patients in Duhok Thalassemia Center is 74. All of them have been recalled. However, only 61 patients agreed to participate in this study.

B) It is unclear how many patients with B-TI are non-transfusion dependent vs. occasional. It is better to add in the text.

Author response: Details of blood transfusion have been added to Table 1. Patients of regular blood transfusion receives >3 blood units of blood per year and those of irregular blood transfusion, including occasional transfusion and non-transfused, receives 0-3 units per year.

C) Serum ferritin and any relation of it with some cardiac parameters in echocardiography (such as LVEDD, LVESD) are not evaluated in these patients.

Author response: The ferritin relation with cardiac parameters, such as PHT and TRV, its indirect relation with cardiac dimensions and volumes, and the difference of serum ferritin and LIC uses have been added. Kindly refer the text to find it: (Though this study had made no data with regard to iron overload in the liver, a significant association between higher ferritin levels and increase in pulmonary artery systolic pressure and higher jet velocity of tricuspid regurgitation had been found. The use of serum ferritin to monitor iron overload is inexpensive and accessible. In TI, serum ferritin assays may underestimate the actual iron load and liver iron concentration (LIC) is more reliable, though invasive).
D) It is mentioned that "molecularly confirmed thalassemia intermedia " but their diagnosis is mostly base on "clinical decision" that is better to add in methods section.

Author response: “molecularly confirmed thalassemia intermedia” has been deleted in order to show that only the clinical diagnosis of cases is depended in Methods.

E) What is the level of significant p- value? It should be added in methods (for example: p value less than 0.05 is considered significant).

Author response: “p value < 0.05 is considered significant” has been added to Methods.

Statistical review: The statistics have been reviewed by an expert at University of Duhok, School of Medicine, Department of Epidemiology. Comparison of categorical counts and percents depend on chi-square test, and comparisons of continuous means ±SD depend on one-way ANOVA test.

Reviewer 2: Dimitrios Tsiapras

1. As reported in the limitations of the study section no healthy controls (sex and age matched as in other studies) have been included to clarify the effects of TI in cardiopulmonary function.

Author response: With all my respect, I have to say here that I kindly do not agree on this point. Our view is that addition of healthy controls, though may be useful, is not necessary as the aim is to see the relation between clinical and echo findings within TI patients of same group in order to explain the accumulative effects of changes in clinical characters with echo parameters. Moreover, some studies on TRV in hemoglobinopathies, including (Pulmonary Hypertension, as a Risk Factor for Death in Patients with Sickle Cell Disease, n engl j med 350; 9 www.nejm.org February 26, 2004. Table 2) have used a similar approach and did not include healthy controls for specific purposes of comparisons of different TRV groups.
2. While increased LA or LV diastolic pressures are discussed as cause of PH the authors have not included not even LV inflow Doppler Echo parameters as an estimation of LV diastolic function.

**Author response:** transmitral inflow sampling by pulsed Doppler for LV diastolic function estimation has been added to Methods, Results and Discussion.

3. As thalassaemic patients are usually underdeveloped all dimensions and volumes have to be indexed to BSA.

**Author response:** The weights, height, BMI and BSA have been taken. Cardiac dimensions and volumes have been indexed to BSA as recommended by the reviewer.

4. Advanced patients age, delay in 1st transfusion, lower regular Hb and less regular transfusions in the patient group with higher TRV are compatible with the pathophysiology of PH in TI as discussed. An explanation of high ferritin levels have to be provided in the group with less blood transfusions during life (delayed onset and less regular BT's).

**Author response:** There are two explanations: 1) the regular blood transfusion means the regular blood transfusion $\geq$3 units/year in the year before the study. Accordingly, it specifically refers to the last year of regular transfusion; 2) Most of patients, especially the group with higher TRV, is not on regular iron chelation. This has been added to the text: (Unsurprisingly, despite of less regular blood transfusion among higher TRV subgroup in the year before this study, relative higher ferritin levels among them was found which is partly attributed to lesser utilization of iron chelation in the same subgroup, the increased risk of progressive ferritin accumulation with advancing age and increased rate of gasrointerstinal iron absorption in TI).

5. The possible cause of exertional dyspnea have to be clarified in the discussion as in the literature provided (as references) there is a clear distinction between LV dysfunction (diastolic or systolic) and prominent right heart dysfunction due to PAH (coming from coagulopathy or elastic tissue abnormalities or NO depletion).
Author response: More details on exertional dyspnea have been added to the discussion section. With regard to LV related dyspnea, the following has been added to the text: (The larger LV dimensions could be explained by the presence of chronic anemia, which is associated with increased blood volume. The lower capacity of the blood to carry an adequate amount of oxygen to peripheral tissues was overcome by the higher cardiac output and the venous return was, therefore; increased. This significant volume overload was carried out through the Frank-Starling mechanism). Regarding RV and PHT related SOB, the following has been added: (The observation that markers of hemolysis are associated with pulmonary hypertension in chronic hemolytic disorders suggests that there is a distinct syndrome of hemolysis-associated pulmonary hypertension. After release of hemoglobin into plasma, the plasma hemoglobin can scavenge nitric oxide as well as catalyze the formation of reactive oxygen and nitrogen species, the processes that can lead to acute and chronic pulmonary vasoconstriction).

6. LV systolic dysfunction (drop of LVEF) in higher TRV patients have also to be discussed. In the papers mentioned (Aessopos et al, Bosie et al and Vaccari et al) there are no data supporting drop of LVEF in TI patients. In the first paper, EF in diseased TI patients is 76% (Tb 5), the second paper deals with Thalassaemia major patients (not TI) and in the third only Thal Major patients showed a drop in LVEF compared to the healthy controls.

Author response: The above indicated paragraph in discussion section has been revised precisely and corrections have been made according to references.

7. Statistical review has to be performed. Even not an expert in statistics I think that Anova test has to be performed to detect differences between more than 2 groups with continuous variables.

Author response: As the reviewer recommended, the statistics have been reviewed by an expert at University of Duhok, School of Medicine, Department of Epidemiology. Comparisons of categorical counts and percents depend on chi-square test, and comparisons of continuous means ±SD, between more than two groups, depend on one-way ANOVA test.
Minor Essential Revisions:

1. A literal review may be needed as some minor mistakes are detected (eg TRV is used in the discussion to define both velocities and pressure gradients).

**Author response:** A literal review has been conducted and, accordingly, corrections have been made.

**Quality of written English:** As recommended by the reviewer to edit the quality of written English. Following the recommendations, the paper has been proofread by an English Language linguist at University of Duhok’s College of Arts.

Many thanks,
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