Reviewer's report

Title: Hepcidin gene polymorphisms and iron overload in β-thalassemia major patients refractory to iron chelating therapy

Version: 1 Date: 14 Oct 2019

Reviewer: Maria Sonati

Reviewer's report:

The revised manuscript is better written and more informative than the one initially submitted. However, there are still some changes to be made. The statement "Beta-thalassemia is the most common group of hereditary hemoglobinopathies" should be replaced by "Beta-thalassemia is one of the most common group of hereditary hemoglobinopathies" in both Abstract and Background. Still in the Abstract, in the phrase "Several studies emphasized the role of single nucleotide polymorphisms (SNPs) located in the promoter region in the expression of the gene.", "in the expression" must be removed; "A total of 102 adult beta-thalassemia major patients" since they are all adults; "All samples were homozygous for allele T of c.-153T&gt;C" is the opposite: all samples were homozygous for allele C (and the mutation is c.-153C&gt;T), according to the main text.

In the Background, the average frequency of beta-thalassemia in Iran, as well as its relationship to malaria, should be commented. The first sentence of the next paragraph (line 23, page 3) can either be removed or should include the introns as well, as many mutations occur in these regions, including IVS-II-1. At the end of the Background, the absence of other similar studies in the country should be emphasized. It is the justification of the work.

In the Results, the 'p' values of the allelic and genotypic frequency analyzes for Hardy-Weinberg equilibrium evaluation should be included in Table 3. The median and the range would more adequately express the serum ferritin levels in Table 2.

In the Discussion, "All patients were homozygotes for c.-153C&gt;T variant" should be replaced by "All patients were homozygotes for the normal allele (c.-153C)", ... (line 28, page 9).

At the end of the Discussion, it would again be interesting to emphasize that this is the first study of its kind in Iran and what is its relevance.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

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