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Short Communication

Evaluation of the Association of Transferrin Receptor Type 2 Gene Mutation (Y250X) with Iron Overload in Major β-Thalassemia

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Abstract

Thalassemia is an inherited blood disorder in which the body produces defective hemoglobin. One of the important processes to reduce the complication of major β-thalassemia is blood transfusion that leads to elevated ferritin levels in the blood. Many patients who have major β-thalassemia may have hemochromatosis conditions resulting from iron metabolism disorders. In patients who have β-thalassemia, the mutation Y250X in the *TFR2* gene may play a role in the incidence of hemochromatosis. This study aimed to determine the relationship between ferritin levels and Y250X mutation in major β-thalassemia patients. In the present study, 12 blood samples were divided into nine major β-thalassemia patients and three healthy controls. The DNA was isolated from blood samples and the amplification of the target region was performed based on the specific primers. Sanger sequencing was used to find genetic single nucleotide polymorphisms associated with iron overload. Blood parameters, such as hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, and serum ferritin levels were analyzed and the recorded data showed the following results: 8.1±0.8 g/dL, 84.6±5.5 fL, 27±0.7 pg, respectively. The recorded data showed that the mean serum ferritin level in major β-thalassemia patients was 1921.7±848 ng/mL. The Y250X mutation was not found in major β-thalassemia patients and healthy controls.

Keywords: β-Thalassemia, Ferritin, Hepcidin, Mutation

1. Introduction

β-thalassemia is one of the most predominant forms of thalassemia in which the production of hemoglobin β chain is impaired quantitatively. β-thalassemia is characterized by anemia and depends on the severity of the anemia. Iron metabolism is a multi-factorial process. This complex procedure is controlled by several mechanisms mainly targeting intestinal absorption. A key element in iron hemostasis is called Hepcidin, which is a small antimicrobial peptide encoded by the hepcidin antimicrobial peptide gene on 19q13. It contains 2637 bp and comprises three exons (1).

Transferrin receptor type 2 (*TFR2*) gene is located at 7q22.1 and produces a single membrane protein type II. The function of *TFR2* is regulating irons that enter the cell by binding once with transferrin protein and hepcidin enzyme according to the concentrations of iron (1).

The Y250X mutation (604720.0001) occurred in a region shared by both the alpha and beta transcripts of TFR2. Moreover, it has been associated with type III hereditary hemochromatosis which was caused by a homozygous nonsense mutation in the *TFR*2 (2).A transversion C/G at position 13451 of Homo sapiens *TFR*2 (accession number: NG_007989.1) lead to amino

acid change tyrosine at residue 250 of the transcript. These mutations form the stop sign (TAG) are symbolized by Y250X (3).

The mutations of the *TFR2* gene may play a role in hemochromatosis in humans. The relationship between*TFR*2 and variations of the erythroid were observed in two points: first, the adjacency of *EPO* and *TFR2* genes that may contribute to joint regulation, and second, the single nucleotide polymorphisms (rs80338880) in the *TFR2* gene was correlated with erythroid quantitative characteristics, such as erythrocytes account and hematocrit (4).

Despite the fact that TFR2 is necessary for effective erythropoiesis, *Tfr2* null mice and *TFR2* hemochromatosis patients have functional erythropoiesis and may sustain several phlebotomy procedures without developing anemia complications(5).Therefore, the current study was designed to analyze the association between ferritin levels and Y250X mutation in major β-thalassemia.

2. Materials and Methods

2.1. Patients

Blood samples were obtained from nine major β-thalassemia patients and three healthy individuals in Samawah city, Iraq. All participants signed informed consent. Inclusion criteria were regular transfusion (2-4 week interval), regular iron chelating therapy, and mean age of above 5 years. Iron overload status was defined as ferritin level more than 1000 ng/ml based on the guidelines of Thalassaemia International Federation.

The diagnosis of thalassemia was based on the clinical manifestation and hemoglobin electrophoresis. The iron level was estimated by serum ferritin, in addition to hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin.

2.2. Molecular Analysis

In total, three mL of Ethylenediaminetetraacetic Acid (EDTA)-blood was lysed with 12 mL of lysing buffer (NH_4CL 150 mM, $NaHCO_3$ 10 mM, EDTA 1 mM) and centrifuged at 3000×g for 10 min. The supernatant was discarded and the white blood cell pellet was washed with normal saline twice. Genomic DNA was extracted from 0.2 mL of white blood cell with a commercial assay (GeneAll® Exgene™ Blood SV mini, Seoul; Korea) according to the instructions of the manufacturer (6).

Analysis of *TFR2* gene Y250X mutation,12 blood samples were subjected to DNA extraction using DNA Wizard Genomic Purification Kit (Promega, USA). Polymerase chain reaction (PCR) amplification of specific target region by GoTaq® G2 Master Green Mix (Promega, USA). The used primers were1 µl of forward sequence 5′TGCACTGGGTCGATGAG′3 and reverse 3′CTCAAGCCCTCCCTCT′5. The thermal profile included one cycle at 95°C for 2 min (initial denaturation), 35 cycles at 95°C for 60 sec (denaturation steps), at 56°C for 60 sec (annealing steps), and 72°C for 60 sec (synthesis), and one cycle at 72°C for 2 min (final synthesis).

The purification of PCR products was performed using a Clean-Up system for PCR products (Wizard SV Gel, Promega, USA) in accordance with the instruction manual of the manufacturer. Genetic Analyzer 3137xl (Thermo Fisher Scientific, USA) was used to electrophoretically separate and detect the purified PCR products according to the instructions of the manufacturer. The DNA sequences quality had been manually inspected in the chromatogram of all samples.

The reference sequence of human *TFR2* with the accession number NG_007989.1 (www.ncbi.nlm.nih.gov) was aligned to the sequencing data by using Sequencer software (versions 6, Gene Codes, USA).

2.3. Statistical Analysis

Statistical analysis was performed on SPSS statistic software (version 23.0) using an independent t-test for the numeric variables and Fisher's exact test for the nominal variables in which some categories had less than 5 cases. Any value less than 0.05 was considered statistically significant.

3. Results

Based on the results of blood parameters, the mean serum ferritin levels were 1921.7±848 ng /ml. Moreover, hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin values were 8.1±0.8 g/dL, 84.6±5.5 fL, 27±0.7 pg, respectively. The PCR product of Y250X mutation in the *TFR2* gene was selected on gel electrophoresis, and the product size was 355bp.

The DNA sequencing was performed on the PCR products. The result of the sequence showed alignment with the sequence of Homo Sapiens*TFR2*, reference sequence gene at 7q22, and accession number of NG_007989.1 which indicated that Y250X mutation was not found in major β-thalassemia patients and healthy control (all sample sequence wild type).

4. Discussion

Iron overload is caused by defects in the iron absorption pathway. Several studies have reported that the mutations in the *TFR2* gene induce increased intestinal iron absorption by increased iron uptake during diet and macrophage iron release leading to tissue iron overload (7, 8). Results of a study have indicated that the effects of Y250X mutation on *the TFR2* gene may elevate iron levels in the blood (9). In major β-thalassemia patients, the blood transfusion dilutes some complications of thalassemia syndrome while raising the iron level (10). This mutation plays an important role in iron hemostasis in the body (2).

In this study, the Y250X mutation was absent in all samples. This result was in agreement with those of a study performed by Sun, Guo (11) which reported that Y250X mutation in the *TFR2* gene does not occur in Tibetan patients with iron overload. Santos, Cancado (12) described that Y250X mutation due to iron overload was not detected in Brazilian blood donors. Moreover, Y250X mutation had effects on clinical manifestations of iron overload. This document searched for the Y250X mutation in 63 individuals who had hereditary hemochromatosis. The results indicated that most of the patients had C282Y and H63D mutations, while the Y250X mutation was not found in any of the 63 individuals that were tested (13).

Several studies reported that Y250X mutation of the *TFR*2 gene plays a role in the homeostasis of iron and abnormal iron absorption. In their study,Kawabata, Fleming (14) found that homozygous *TFR*2 (Y245X) mutant mice (mirror to the *TFR*2[Y250X]mutation in humans) showed a hereditary hemochromatosis phenotype. Fleming, Ahmann (15) affirmed the essential role of Y250X mutation in iron homeostasis and stated that it may be considered a prognostic marker of abnormal iron absorption. Piperno, Roetto (16) performed a study on two Italian patients with homozygous Y250X mutation, both of whom had increased iron concentration in hepatic cells and elevated serum ferritin.

In conclusion, based on the above-mentioned results, it can be said that that there is no association between *TFR2* gene mutation (Y250X) and iron overload in patients with major β-thalassemia.

Authors' Contribution

Study concept and design: J. A. J.
Acquisition of data: N. A. M. A.
Analysis and interpretation of data: J. A. J.
Drafting of the manuscript: N. A. M. A.
Critical revision of the manuscript for important intellectual content: J. A. J. and N. A. M. A.
Statistical analysis: N. A. M. A.
Administrative, technical, and material support: J. A. J.

Ethics

All studies were performed in compliance with the rules of humane treatment of Al Muthanna University, Samawah, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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