Effect of gene FOXO3 on women with Thalassemia major in the city of Diwaniyah

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Abstract

The current study was conducted in the Department of Life Sciences / Faculty of Science / Al-Qadisiyah University and in collaboration with the Women's Hospital and the Center for Hematology in Diwaniyah for the period from 1/10/2017 to 1/4/2018. A sample of women was divided into the first two groups (30) of whom were women with thalassemia and the second included 20 (20) non-thalassemia women.

The biochemical tests of the Antioxidant (Catalase) (CAT) showed a significant decrease of P <0.01 in parameter of the group of patients compared with control.

Results of gene expression showed a marked decrease in expression of the FOXO3 gene for the group of infected women compared to control.

The results of the present study conclude that women with Thalassemia major type β had a clear effect on public health, especially on reproductive efficiency of women, as well as a cycle in reducing the level of gene expression of FOXO3 gene responsible for oxidative stress resistance in cells.

Keyword :Thalassemia , Oxidative stress ,Catalase ,Antioxidant , FOXO3

Introduction

Thalassemia is a genomic genetic disorder that occurs in the gene responsible for the synthesis of Globine in the red blood cells, resulting in a malfunction of one or more of the chains of the globine, and thus leads to a failure to manufacture Hemoglobin (Hb) responsible for the transfer of oxygen to different parts of the body, there are two types of thalassemia: α and β, where beta-thalassemia is the most common of the hereditary blood diseases that cause a deficiency of β

+ or the absence of β (β) in manufacture of betaglobine chains within the quadruple hemoglobin chains, Which consists of two chains α and two β chains [1] . Thalassemia is the most common genetic disorder in the world, not just Iraq, and the anatomical and histological changes observed in thalassemia reflect anemia severe hemolytic and chronic hemochromatic dysfunction wheating about a lack of oxygen [2] . The disease is characterized by varying degrees of inefficiency blood, chronic anemia, hemolysis [3] , [4].

The oxidative stress is important type of stress which is defined as a state of imbalance between oxidants and antioxidants, which later works on cell breakdowns through effective reactive oxygen species ROS [5] . Antioxidants can be defined as a substance that when present in a few concentrations compared with oxidizing essentials, works to remove or inhibit the oxidation process of the basic material. It have the ability to give electrons and convert free radicals into stable compounds that are unable to interact with biological molecules and thereby eliminate the harmful activity of free radicals [6] [7] . The most important oxidative damage is on the red blood cells caused a process of programmed cell death and inefficient manufacturing of RBC [8] [9] where apoptosis is a form of cell death characterized by cell contraction, chromatin condensation, nucleicysis, dysfunction of the plasma membrane and thus cell dissolution [10]. Excess iron is one of the causes of oxidative stress in Thalassemia patients , the iron-forming hematopoietic molecule is a water-damaging molecule that can interact with the fat and proteins this will lead to oxidative reactions and has the ability to bind to enzymes receptors and transcription factor,cell function change, metabolism and gene expression this can lead to DNA oxidation and scaling of mitochondrial function as well as it negatively affects the activity of the antioxidant enzymes [11] . ROS which is the result of an increase in iron in the body [8]. Thus oxidative stress (OS) occurs, which acts on oxidation of cell membranes. This leads to the emergence of some toxic spinous products To undermine cellular functions [12] .

. The antioxidant defense mechanisms are divided into two types: either enzymatic or non-enzymatic, both of which act to inhibit the harmful effects of oxidants antioxidants directly remove ROS, the most important of which are superoxide dismutase (SOD), Catalase (CAT), non-enzymatic systems such as GSH, URICACID or food-produced products such as vitamin C and vitamin E [13] . Catalase (CAT) is an enzymatic antioxidant that decomposes hydrogen H_2O_2 into oxygen and water, thereby reducing the harmful effects of H_2O_2 [14].

FOXO3 is defined as one of the factors responsible for cloning in the cell multiplication cycle, the main gene responsible for aging Its association with longevity in many organisms is located on chromosome 6 [15] , its belongs to a large genetic family and is one of the transcription factors that play an important role in cell proliferation, cellular differentiation, programmer cell death, DNA repair and late cell carcinogenesis [16] . FOXO3 is also an important gene for building Red blood cells (RBCs) and their membranes, both in the stages of formation of pellets precursors or later, contribute to inhibiting the roots of the oxygen interactive (ROS) and prevent them from performing the destructive work of antioxidants, which is characterized by its ability to affect the effect of the production of oxidants [12]. Its responsible for regulating a number of physiological processes such as apoptosis and response to oxidative stress [17] . FOXO agents generally regulate the expression of detoxification enzymes, which are antioxidants such as SOD,

Catalase [16]. Its responds to the process of oxidative stress by stopping the cellular replication cycle and reproducing the damage to the DNA caused by oxidative stress , FOXO3 deficiency leads to reduced cell regeneration [18] and to generation Erythroid precursors [19] ,demonstrate that FOXO3 activation leads to resistance to oxidative stress in most cells of the body [20] .

Methods

The present study has been carried out at the Women's and Children's Hospital and the Hematology Center in Diwaniyah during period extended from 1/10/2017 to 1/4/2018. 30 samples of women with Thalassemia major and 20 samples of women Slims as control group, the age of states 14 – 45 years .

Collection of samples

Samples were collected from Thalassemia Center in Diwaniyah as follows:

First, 2 ml of venous blood was withdrawn by a disposable syringe and then placed in special EDTA tube for molecular tests where it was left in freezing under -20° C

Second: 4 mL was withdrawn in gel tubes and left at room temperature for 20 minutes for coagulation and then placed in centrifuge at 3000 RPM for 10 minutes. These samples were kept in a continuous freezing under (-20 °) until they were used as (Eppendrof Tube) , for Antioxidant enzymatic Catalase (CAT) .

The control group was taken 20 healthy cases and the samples were collected from the Faculty of Science / University of Qadisiyah and in the same way as the samples collected with patient samples .

Method of Measurement of antioxidant catalase (CAT)

Catalase is one of the catalysts or accelerators for the analysis of hydrogen peroxide, which is one of the strong oxidants. Therefore, the measurement of CAT's effectiveness is determined by the measurement of hydrogen peroxide decomposition the method depend on aebi method [21] [22] .

Molecular assays used to determine the gene expression level of FOXO3 gene

Measuring the level of gene expression (reverse transcription)

The level of gene expression is measured using the reverse transcription method to measure the amount of transcribed levels (mRNA) to denote the gene expression of a gene (FOXO3 gene) and the use of the conservative gene as a standard regulator gene to calculate the amount of gene expression

The RNA is extracted using the Trizol kit, supplied by Korean company Pioneer, according to the company's steps .

The extracted RNA is detected using a special nanodrop spectrophotometer by determining the concentration of RNA ng \ µl and measuring RNA purity by reading the absorbance at 260/280 nm .

The cDNA synthesis method was used by using RNA samples extracted using Accupower Rockscript Premix. Depending on Company steps .

Primers

The prefixes are designed using NCBI GenBank Data and using Primer3 plus. This prefixes are equipped by Korean Pioneer Company as Table 1

TAPLE 1 : The Primers used for FOXO3 and GAPDH gene .

Primer	Sequence		Amplicon
FOXO3	F	TTCCGTTCACGCACCAATTC	73 bp
	R	ACTCTGTGCTTGCCATGATG	
GAPDH	F	TCAGCCGCATCTTCTTTTGC	122 bp

NCBI Reference Sequence for FOXO3 : NM_001455.3

NCBI Reference Sequence for GAPDH : NM_002046.5

Statistical Analysis

The data were analyzed using prism (SAS Institute,Inc,USA) pad graph Version 5 The data were analyzed using a test application the T-Test and the Chi-square test was applied for this purpose e. At the probability level of ($P<0.01$) [23] .

The results of the gene expression were statistically analyzed using the one way ANOVA LSD method at a probability level of 0.05% using the SPSS statistical program.

Results and discussion

The results of the present study showed a significant decrease ($P <0.01$) in the level of the anti-oxidant CAT in women with thalassemia compared to the control group as shown in Table (2):

Table 2 : The level of catalase in thalassemia- women compared to the control group

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average	±Standard error	Groups
1.85	± 0.06	Patients
3.82	± 0.22	Control
9.836		t-test

This can be attributed to the disruption of enzymes associated with cell membranes, including CAT. The increase in iron ratio as reported in the results of this study leads to the production of free radicals play a large role in the low percentage of antioxidants, including CAT with the emphasis that this decline has nothing to do with disease Thalassemia is associated with an increased complication. Thalassemia is a hereditary disease resulting from a defect in the hemoglobin chains. The spread of CAT in the cells found in large amounts in RBC is responsible for detoxification of H_2O_2 in cells [24] . [20] confirmed that Increased oxidative stress has a relationship to the generation of free radicals through the surplus of alpha chains compared to beta chains and iron accumulation and the reduction of concentrations of hemoglobin than the normal limit in patients and the increase in oxidative damage associated with the reduction of antioxidants especially catalase is due to the generation of free radicals coming from surplus chains Alpha due to the lack or decrease of beta chains during the RBC formation phase. [25] attributed the reduction of catalase in the results of its study to the destructive effect of free radicals on the catalase protein found on red blood cells. That the decrease in CAT is due to increased oxidation of fat from MDA, which can be CROSS LINK leads to the inhibition of several antioxidants and prevent them from binding to cell membranes, as indicated [26] . The breakdown of oxidants of RBC precursors can cause accelerated RBC programmed death and accelerate the maturation of RBC cells or are inefficient in their function [8] explained the results of their study. [27] showed that the increase in iron ions following the FENTO-HARBER WEISS interaction, which generates ROS, [28] attributed the increase in ROS in large quantities due to the length of the non-balancing period Between oxidants and antioxidants ,[29] stressed that excess iron is one of the causes of oxidative stress in patients with thalassemia, which is considered to be one of the free radicals.

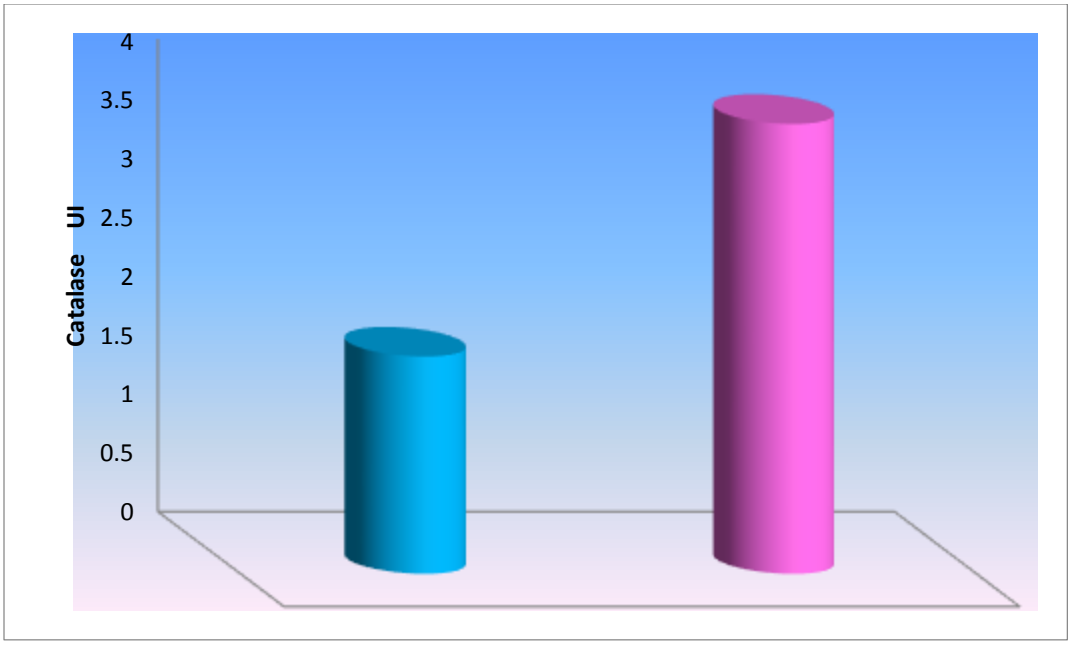

Fig1 Level of CAT in patient comper with control group.

The genetic expression of FOXO3 gene was studied by Rt-PCR technique to determine the amount or amount of gene presence in thalassemia patients compared to GAPH as control gene and then comparing the results of patients with control group results. The results showed a significant decrease in the level of gene expression of FOXO3 in patients compared with group Control as shown in the table (3)

Table 3 The level of gene expression of FOXO3 in patients compared with group Control

Fold change Mean ± SE	Groups
1.626± 0.31	Patients
7.608 ±0.92	Control
7.069	t-test

The decrease in gene expression of FOXO3 may be due to the spread of FOXO3 on the membranes of RBC cells. Since these cells suffer from dysfunction and decay at an early stage of formation, and there is a defect in the manufacture of RBC membranes. The study, which is usually

due to the inability of enzymatic and non-enzymatic defenses to inhibit and remove ROS, thus exacerbates the oxidative stress on RBC and its membranes. The reduction of the gene leads to the lack of genetic expression of antioxidant enzymes which are sweeping to ROS such as CAT and SOD and thus increase the damage of oxidative stress on the cellular membranes and their contents. The loss of FOXO3 also impairs the process of mitotic division in the Erythroid precursors. This inevitably leads to a decrease in the maturation of the precursors erythropiosis stage in the bone marrow. However, activation of FOXO3 during the process of red blood cells plays an important role in the development of hematopoietic stem cells. This explains FOXO3 dysfunction and deficiency in thalassemia patients. Stem cells are mainly suffering from hemoglobin dysfunction and reduced cell division in thalassemia patients FOXO3 gene expression All results of the studies have approximated any abnormalities in FOXO3 Different stages of blood stem cell formation lead to a defect in later RBC formation .

The accumulation of HEM from Erythroide cell mutation makes the cells vulnerable to oxidative stress and this makes the defense mechanisms at the cloning stage worse, thus reducing the efficacy and quantity of FOXO3 and the low level of FOXO3 gene expression. It is possible to have no indication of decreased genetic expression of ROS scanners or dehumidifiers such as CAT , And that the accumulation of ROS may work to expose the cells to crash especially RBC result of CAT decline and that the lack of red blood cells precursors due to the decrease gene expression of FOXO3 gene in patients and that the decrease of the gene significantly affects the organization of the cycle of cell division and maturity of RBC precursors as well as M And the defect in the FOXO3 or deformation of its structure will cause malformation and deformation in

The manufacture of RBC (in addition to the hemoglobin dysfunction), and the death of the mice after removal of the gene or even a defect in the gene and this is referred to as [30] in his study and explained that this is due to the effect of oxidative stress on organs and devices mice that could not resist (OS) Oxidative stress due to loss of gene (FOXO3), and that the maintenance of highly regulated mechanisms to control ROS levels is necessary In order to achieve cellular equilibrium where these mechanisms will subsequently work on low oxidative stress .

FOXO3 loss results in an accumulation of DNA damaged by OS in hematopoietic cell precursors, where FOXO3 is described as a protective gene for the stability of hematopoietic cells and their precursors [31] [32] ..

The loss of FOXO3 stimulates the discontinuation of cell division in RBC precursors, which inevitably leads to a reduction in the maturation of Erythroid, in contrast to the control group, which has a high arsenal of antioxidants that act painfully against oxidants and specifically against the free radicals generated by the accumulation of iron for the increased transfusion of patients Free-radical generation is also the result of early RBC decay with all associated increases in the amount of iron in the blood and this is what [30] base in interpreting the results of his study.

The inclusion of the promoter of the catalase on several sites for association with FOXO3 explains the relationship of gene reduction with CAT declin , This is indicated by [16] , where he confirmed that the activity of Erythroide cell containing several sites for FOXO3 is higher than

that [33] states that the cellular response sites of OS in Erythropiosis contain clonal factors, the most important of which is FOXO3, which controls RBC maturation pathways and OS levels.

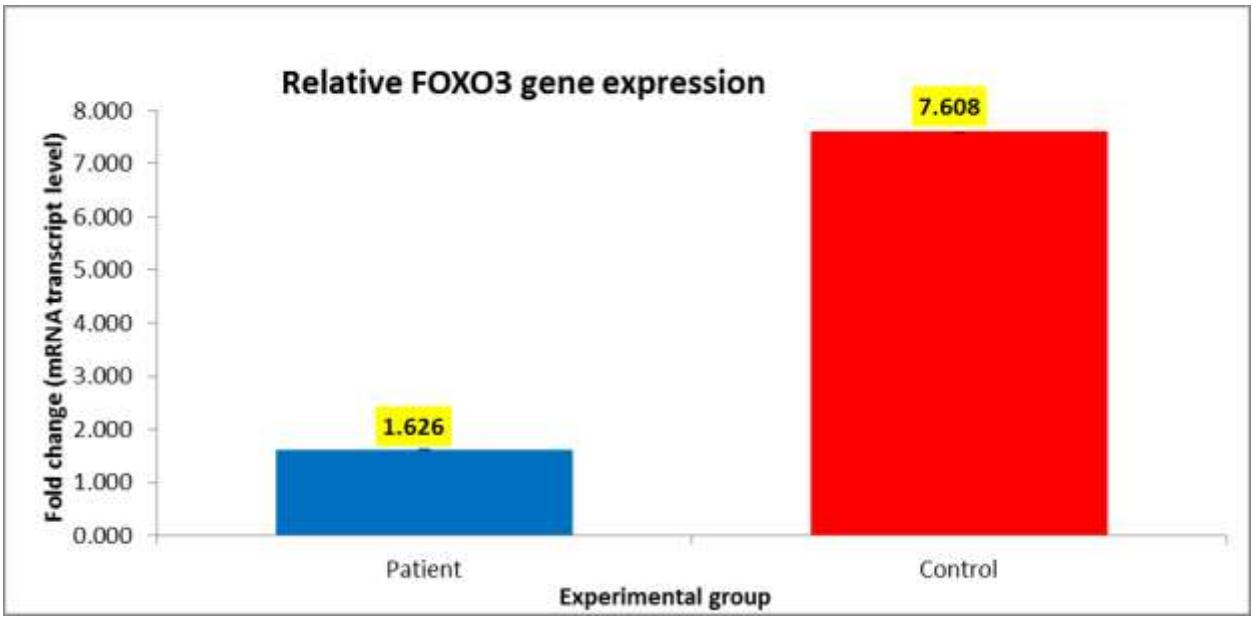

Fig (2)The low level of gene expression of FOXO3 gene for the group of women with thalassemia is significant compared to the control group

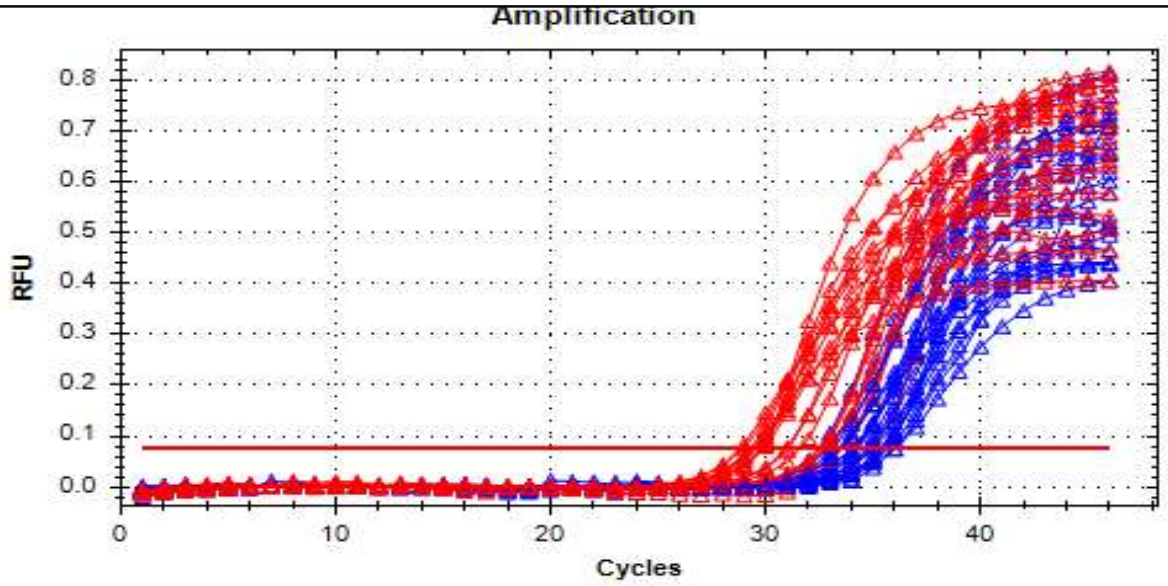

Fig 3 Real – time PCR plote for FOXO3

Where the blue color refers to the patient's sample and red color to .control

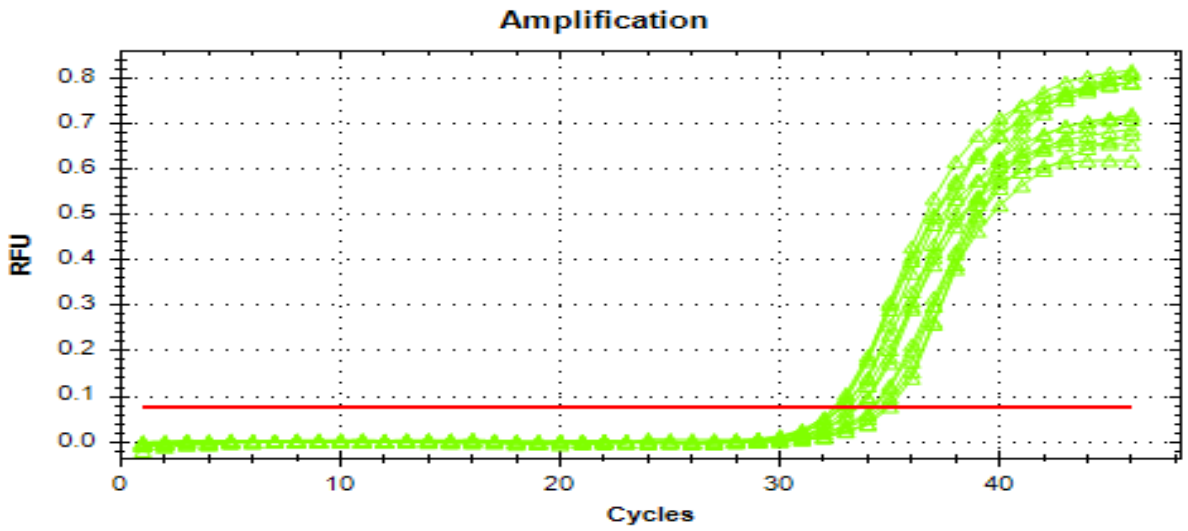

Fig4 Real – time PCR plote forGAPDH

The green color refers to the GAPDH gene change curve

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