Iraqi Journal of Biotechnology, 2022, Vol. 21, No. 2, 579-593

Evaluation of AHSP Gene Expression and Its Serum Level in Iraqi β-thalassemia Major Patients

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Received: 1/6/2022 Accepted: 11/10/2022 Published: December 20, 2022

Abstract: β-thalassemia major is a genetic disorder of hemoglobin production that results in a diminished rate of synthesis of one or more of the globin chain causing variable degrees of anemia. The protein AHSP is an alpha-globin-specific chaperon that has an impact on disease severity in patients with β-thalassemia. studies on the disease markers are still lacking in Iraq. In the recent study, the real time-polymerase chain reaction (RT-PCR) and ELISA techniques were used to investigate and measure the AHSP gene expression in β-thalassemia patients and control, as well as to measure the expression of α and β globin genes in β-thalassemia patients, and to evaluate the serum concentration of A1pha hemoglobin stabilizing protin (AHSP) in patients and the control group. This study was conducted on 90 β-thalassemia patients and 60 healthy as a control group. Blood samples were obtained from the thalassemia patients in Wasit Center / Wasit governourat for Hereditary Anemia for the period from August 2020 to January 2021. The results showed a decrease in AHSP expression in β-thalassemia patients compared with the control. The human AHSP concentration estimated by ELISA appeared to have a significant decrease in the level of AHSP in the serum in β-thalassemia patients compared with the control. In conclusion, AHSP expression is limited and acts as a disease modifier in β-thalassemia. The identification of novel targets will improve the management of this disease.

Keywords: β-Thalassemia; expression; AHSP gene; ELISA, Iraq.

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Introduction

Thalassemia is a complex of various hereditary disorders of hemoglobin combination featuring insufficient production of at least one of the globin chains driving imbalanced globin-chain production, damaged hemoglobin eventually causes anemia (1). β-thalassemia is a recessive hereditary autosomal monogenic disorder caused by point mutation or mild deletions of the 11 βHb genes (HBB) allele, leading to a loss or loss of βHb. As a result, there is a constant overload of incomparable αHb subunits that is very close to binding and contributes to aggregation and precipitation in patients with β-thalassemia. Subsequently, aggregated αHb causes oxidative damage via ROS and encourages RBC degradation (2).

A highly expressed protein called alpha hemoglobin stabilizing protein (AHSP) can act as a chaperone for free chains and prevent their precipitation. It is known as an alpha-hemoglobin (α-Hb) chaperone which specifically recognizes the a-Hb G and H helices, forming a steady complex with free α-Hb till it binds its b-subunits partner (3). If the AHSP gene is functional in thalassemia patients, AHSP binds to αHb subunit and prevents it from aggregating and precipitating (4). However, the condition deteriorates if AHSP gene mutations co-exist in β-thalassemia patients (5).

Experimental evidence has been suggesting that AHSP can be a potential modifier of β-thalassemia via compensatory mechanisms in erythroid precursors and can be a part of therapeutic strategy ameliorating *α*Hb toxicity and the clinical severity of the disease(6). Although AHSP expression links to the severity of beta-thalassemia, its function as a potential genetic modifier of disease severity, has not yet been recognized, therefore, this research aimed to explore AHSP gene expression and its relation with alpha and beta-globin genes expression in a sample of β-thalassemia Iraqi patients.

Materials and methods

This study was conducted during the period from August 2020 to January 2021. All the study experiments were performed at the Institute of Genetic Engineering and Biotechnology for the Postgraduate Studies / University of Baghdad as well as the laboratories of Wasit Centre for Heredity Anemia/ Wasit governourat.

Ethical approval and participants' consent

This study was approved by the council of the Institute of Genetic Engineering and Biotechnology / the University of Baghdad. Signed written consent was taken from each individual participating in the study.

The study design, sample size, and selected criteria or

Samples were selected in this study included B-thalassemia major patients from Wasit Centre for Hereditary Anemia diagnosed as B-thalassemia major.

The selected ninety B-thalassemia major patients with 5-22 age range included 48 males and 42 females diagnosed with Hb electrophoresis, complete blood count had been diagnosed by the center's physicians and sixty apparently healthy individuals in the control group included 34 males and 26 females. The personal information for each patient and control was obtained by a special questionnaire form.

Excluded criteria

Patients group received therapy for thalassemia were excluded from this study, as well as patients with Hepatitis B and C and splenectomized patients were also excluded.

1- Primers

Table (1) shows the primers and probes used in the study.

Table (1): The probes and primers used in this study

Primer	Sequence (5′→3′ direction)	References
Alpha-globin		
Forward	AACTTCAAGCTCC TAAGCCACTGC	Ahmed *et al*, (3)
Reverse	CGAG-GCTCCAGCTTAACGGTATTTG	
Beta-globin		
Forward	ATCCTGAGAACTTCAGGCTCCTGGG	
Reverse	GAGCTTAGTGATACTTGTGGGCCAG	
AHSP		
Forward	CCTGTTAGACCTGAAGGCAGATGGC	
Reverse	AGTCCTCCACCACAGTCACCA TGT	
Beta-actin (Hanse keeping gene)		
Forward	CCACACTGTGCCCATCTACG-3	
Reverse	CCGTGGTGGTGAAGCTGTAG	

Methods

1- Blood Sample collection

Blood sample (5) ml was obtained from each individual in each group by venipuncture using disposable syringes, blood was placed directly into EDTA anticoagulant tubes for Hb electrophoresis and complete blood count (CBC), and to evaluate the AHSP gene expression by quantitative real-time PCR (qRT-PCR).

Two ml of blood without EDTA was used to measure the serum level of AHSP by the ELISA kit.

2- RNA extraction from blood samples

The RNA extraction from whole blood of both patient and healthy control subjects was done by using the protocol in *EasyPure*® Blood Genomic RNA Kit (Transgen, China) according to the company's instructions.

Then, RNA concentration and purity were measured by Nanodrop. The nucleic acid concentration and purity ratio are automatically calculated by the NanoDrop spectrophotometer and the results are ranged between (30- 120 ng/µl) and (1.8-2) respectively.

3- One step gDNA removal and cDNA synthesis

The cDNA synthesis was subjected by using the protocol in *EasyScript*® One-Step gDNA Removal and cDNA Synthesis SuperMix (Catalog No.AE311).

Incubation

For random primer, it was incubated at 25°C for 10 minutes, and for Anchored oligo $(dT)_{18}$ primer and GSP**,** incubated at 42°C for 15 minutes (for qPCR) incubated at 85°C for 5 seconds to inactivate enzymes as shown in table (2).

Table (2): Thermal cycler steps

	Step1	Step2	Step3
Temperature	25°C	42°C	85°C
Time	10min	15min	5seconds
	Random Primer (N9)	Anchored Oligo(dT)18	Inactivate reverse transcriptase enzyme

TransStart® top Green qPCR SuperMix

This procedure was carried out in a reaction volume of 20 µl according to the manufacturer's instructions.

Quantitative (qRT–PCR)

The expression levels of the AHSP gene were estimated by qRT-PCR. To confirm the expression of the target gene, a quantitative real-time qRT-PCR SYBR Green assay was used. Primer sequences are shown in table (1).

The mRNA levels of endogenous control housekeeping gene were amplified and used to normalize the mRNA levels of the housekeeping was prepared according to gene primers sequences are also shown in table (1).

Primer preparation

After dissolving the lyophilized primers in nuclease-free water according to the manufacturer to make a stock solution with a concentration of 100 µM for each primer, a primer working solution was prepared and stored at (-23°c). Diluting 10µL of primers stock solution in 90 µL of nuclease-free water yielded a working solution with a concentration of 10 µM, which was stored at (-23°C) until use.

The Qiagen Rotor gene Real-time PCR System with qPCR soft software was used for QRT-PCR. Using the TransStart® Top Green qPCR SuperMix Kits components, the gene expression levels and fold change were quantified by measuring the threshold

cycle (Ct). Every reaction was performed in duplicate, and negative controls included a non-template control (NTC), a non-amplification control (NAC), and a non-primer control (NPC).

Housekeeping (β-actin) gene amplification:

As an internal control, the housekeeping gene was used to calculate the Ct value. A qPCR reaction for housekeeping gene amplification was performed using the thermal profile shown in table (3).

Table (3): Thermal profile of gene expression

Step	Temperature	Duration	Cycles
Enzyme activation	94ºC	30 Sec	Hold
Denature	94ºC	5 Sec	40
Anneal/extend	62ºC	30 Sec	
Dissociation	55 ºC-95 ºC		

Real-Time qRT-PCR analysis of genes expression

TGenes are used in the calculation of the Ct value. A qPCR reaction for gene amplification was performed using the Thermal profile shown in the table(4).

Table (4): Thermal profile of AHSP, α-globin, and β-globin gene expression.

Step	Temperature	Duration	Cycles
Enzyme activation	94ºC	30 Sec	Hold
Denature	94ºC	5 Sec	40
Anneal/extend	68ºC	30 Sec	
Dissociation	55 ºC-95 ºC		

Statistical analysis

The Statistical Analysis System-SAS (7) program was used to detect the effect of different factors on study parameters. T-test and Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significantly compare between means. The Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability. Estimate the correlation coefficient between variables in this study.

Results and discussion

Molecular analysis

Transcriptomic RNA extract for the blood samples

Total RNA was successfully extracted from all samples. The concentration of total RNA was 82±11 ng/ µl in the patients' group ranging from 68 to 97 ng/µl and in the control group; it was 83±12, the concentrations and purity of total RNA are shown in tables(5).

Table (5): Concentration of total RNA

Group	Total RNA concentration (Mean ± SD)	Range ng/ µl
Group 2 : Patient (no.= 90)	82±11	68-97
Group 1 :Control (no.= 60)	83±12	71-98
T-test	7.931 NS	---
p-value	0.602	---

The purity of total RNA samples was 2.08±0.04 ng/µl in the patients' group ranging from 1.98 to 2.19 ng/µl and in the control group; it was

2.07±0.05 ranging from 1.96 to 2.19 ng/µl, the concentrations and purity of total RNA are shown in table (6).

Table (6): Purity of total RNA sample

Group	Purity Total RNA (Mean ± SD)	Range
Group 2 : Patient (no.= 90)	2.08±0.04	1.98-2.19
Group 1 :Control (no.= 60)	2.07±0.05	1.96-2.19
T-test	0.317 NS	----
p-value	0.294	---

Complementary DNA (cDNA) synthesis

cDNA reverse transcription was conducted on the second day of RNA extraction. A common primer reaction was applied since it was needed to have cDNA for all the genes in the study and housekeeping genes.

The efficiency of cDNA concentration was evaluated using qPCR, and all steps were associated with perfect yield, indicating efficient reverse transcription.

Gene expression

In the current study, gene expression was measured using (RT-qPCR), a fluorescent dye that recognizes any double-strand DNA, including cDNA, and the amplification was recorded as a Ct value (cycle threshold) .The presence of more copies of the target was indicated by a lower Ct value and vice versa.

In terms of gene expression, the highest value indicates low gene expression and a low Ct value indicates high gene expression (8,9).

Real-time PCR quantification of β-actin expression

Housekeeping genes are widely used as internal fertile for gene expression normalization for western blotting, northern blotting, RT-PCR, (10). A good correlation between gene expression values was obtained when using β-actin as a reference gene(11).

The Ct value of *β-actin* and the housekeeping gene used in the present study is shown in table (7). The Ct value was (15.490 ± 1.25) in the control, its range was (15.39-15.55), and the Ct value was (15.492±1.39) in the patients and the range (15.01-15.68).

No significant difference was found between these groups regarding the mean Ct value of *β-actin*.

Table (7): Comparison between different groups in CT value of β-actin.

Group	No.	Mean ± SD of Ct value	Range
Group: Patient	90	± 1.3915.492	15.01-15.68
Group: Control	60	± 1.2515.490	15.39-15.55
T-test	---	1.882 NS	---
P-value	---	0.738	---
NS: Non-Significant.			

The 2^{-Ct} value in the control group was (2.173E-05) and in β-thalassemia patients it was (2.17E-05). The computed ratio for gene fold expression was 1.00 and 0.998 respectively. These small variations in gene fold expression between the study groups render the *β-actin* gene a useful control gene, as shown in table (8).

Table (8): Comparison of *β- actin* Fold expression between study groups

Group	Means Ct of *B-actin*	2^{-Ct}	Experimental group/ Control group	The fold of gene expression
Group: Patients	15.492	2.17E-05	2.17 E-05/2.173E-05	0.998
Group: Control	15.490	2.173E-05	2.173E-05/2.173E-05	1

The amplification plots and dissociation curves for each run were recorded, figure(1) shows the amplification and dissociation curves for the β-actin gene.

The β-actin gene encodes a cytoskeleton structural protein and is possibly the most widely used gene for normalization in gene expression experiments (12).

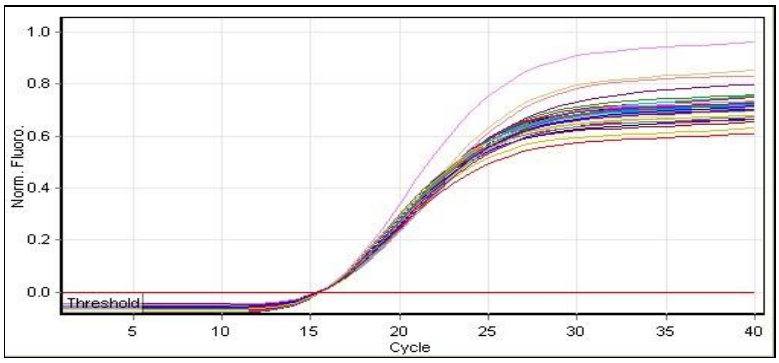

Figure (1): *B actin* amplification plots by qPCR samples included all study groups. The photograph was taken directly from the Qiagen Rotor gene Q 6000 qPCR machine

Real-time PCR quantification of *AHSP* expression

The AHSP gene was located on human chromosome 16 and the structure of AHSP mutation leading to its reduced expression or altered function provides evidence that AHSP could modulate β-thalassemia (13).

Results of real-time PCR quantification of AHSP gene expression are shown in table(10).

The Ct value of AHSP is shown in table (9). The Ct mean value was (20.82) in the control, the Ct mean value for patients was (19.92), the fold gene expression for the control group was 1.00 and for β-thalassemia patients group was (1.868).

There is a statistically significant decrease in gene expression of Ct AHSP, Ct β-globin, and Ct α-globin in β-thalassemia patients compared with the control group but Ct β-actin appeared non-significant difference between patients and control.

The hypothesis is that AHSP may act as a genetic modifier in β-thalassemia and this is proven by (14,15).

Reduced AHSP mRNA expression has been associated with clinical variability in some cases of β-thalassemia, it has been shown that α-Hb variability may also impair AHSP-αHb interaction (16).

The association between reduced AHSP mRNA expression and a more severe phenotype among individuals with identical β-thalassemia (17).

Table (9): Fold of AHSP expression depending on 2-$^{\Delta Ct}$method

Groups	Means Ct of *AHSP*	Means Ct of *B actin*	ΔCt (Means Ct of *AHSP* - Means Ct of *B actin*)	2-$^{\Delta Ct}$	experimental group/ Control group	The fold of gene expression
Group: Patient	19.92	15.492	4.428	0.0464557	0.0464/0.0248	1.868
Group: Control	20.82	15.490	5.33	0.0248605	0.0248/0.0248	1

The Ct value of AHSP used in the present study is shown in table (10). The 2-ΔΔCt mean value was (20.82) in the control, and the 2-ΔΔCt mean value for patients was (19.92), the fold gene expression for the control group was 1.00 and for β-thalassemia patients group was (1.868).

Table (10): Fold of *AHSP* expression depending on 2-ΔΔCt method.

groups	Means Ct of *AHSP*	Means Ct of *B actin*	ΔCt (Means Ct of *AHSP* - Means Ct of *B actin*)	Mean ΔCt Calibrator (ct *AHSP*- ct *B actin*	ΔΔCt	2-ΔΔCt	experimental group/ Control group	The fold of gene expression
Group: Patient	19.92	15.492	4.428	8.71	-4.282	19.45	19.45/10.41	1.868
Group: Control	20.82	15.490	5.33	8.71	-3.38	10.41	10.41/10.41	1

Each run's plot, including the amplification plots and dissociation curves, was recorded, figures (2) and (3) show the amplification and dissociation curves for the AHSP gene.

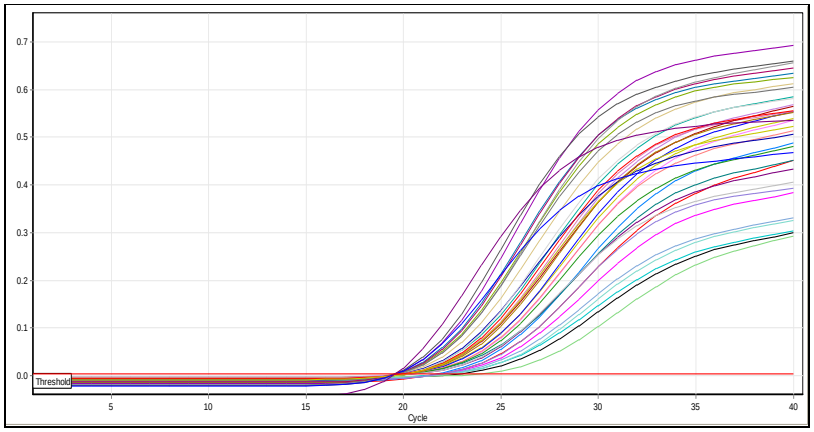

Figure (2): All study groups were represented in the AHSP amplification plots by qPCR samples. The image was captured using the Qiagen Rotor gene Q 6000 qPCR machine.

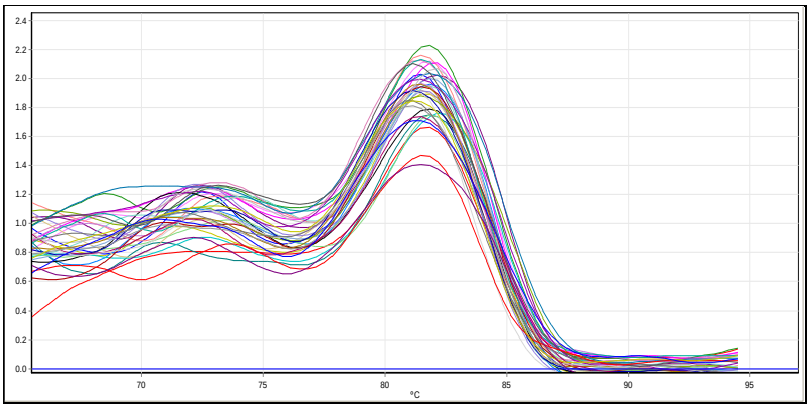

Figure (3): *AHSP* dissociation curves by qPCR Samples included all study groups. Melting temperature ranged from 81°C to 83°C. The photograph was taken directly from the Qiagen Rotor gene Q 6000 qPCR machine.

The AHSP gene structure was determined by nuclear magnetic resonance spectroscopy (NMR) and X-ray crystallography (13).

Impaired AHSP was also detected to be associated with the development of β-thalassemia (2).

Results of this study were agreed with (18) because the observed AHSP gene expression decreased in mild as compared with moderate and severe β-thalassemia, The AHSP helps in the stabilization and solubility of α-globin chains to avoid ineffective erythropoiesis by forming a stable but reversible complex with free α-globin chain (19), also AHSP prevents the toxic effect of α-globin by hindering it precipitation and enhancing its thermal stabilizing, this function is performed by enhancing proper folding of the α-Hb, inhabiting auto-oxidation of holo α-Hb, as well as refolding of any denaturation protein (18).

Real-time PCR quantification of β-globin expression

β-thalassemia is an autosomal disorder characterized by a genetic defect in the synthesis of the α-globin chain in hemoglobin. A lack of β-globin chains causes intracellular precipitation of excess -globin chains, resulting in ineffective erythropoiesis. In general, a faulty β-globin gene results in a reduction (β+) or absence (β°) of the gene product (20).

The Ct value of betα-globin and the housekeeping gene used in the present study is shown in table (11). The Ct mean value was (15.40) in the control, the Ct mean value for patients was (13.73), the fold gene expression for the control group was 1.00 and for the β-thalassemia patients group was (3.186).

Table (11): Fold of β-globin expression depending on 2-ΔΔCt Method

Groups	Means Ct of β-globin	Means Ct of *B actin*	ΔCt (Means Ct of β-globin - Means Ct of *B actin*)	Mean ΔCt Calibrator (ct B g. -ct *B actin*	ΔΔCt	2-ΔΔCt	Experimental group/ Control group	The fold of gene expression
Group: Patients	13.73	15.492	-1.762	2.042	-3.804	13.967	13.96/4.38	3.186
Group: Control	15.40	15.490	-0.09	2.042	-2.132	4.383	4.38/4.38	1

The Ct value of betα-globin and the housekeeping gene used in the present study is shown in table (12). The 2-ΔΔCt mean value was (15.40) in the control, and the 2-ΔΔCt mean value for patients was (13.73), the fold gene expression for the control group was 1.00 and for β-thalassemia patients group was (3.186).

Table (12): Fold of betα-globin expression depending on the $2^{-\Delta Ct}$ method

Groups	Means Ct of β-globin	Means Ct of *β actin*	ΔCt (Means Ct of β g. - Means Ct of *β actin*)	$2^{-\Delta Ct}$	Experimental group/ Control group	The fold of gene expression
Group: Patient	13.73	15.492	-1.762	3.3916799	3.391/1.064	3.186
Group: Control	15.40	15.490	-0.09	1.0643702	1.064/1.064	1

The plot of each run was recorded including the amplification plots and dissociation curves – Figures (4) and (5) show the amplification and dissociation curves for the beta-globin gene.

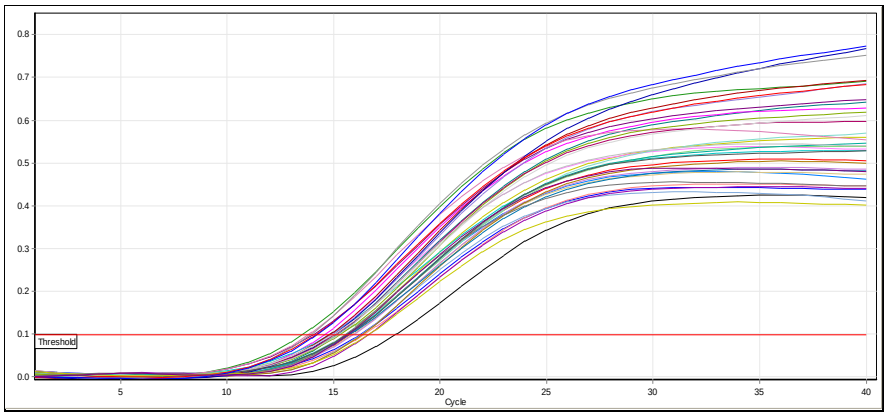

Figure (4): Beta-globin amplification plots by qPCR samples included all study groups. The photograph was taken directly from Qiagen Rotor gene Q 6000 qPCR machine.

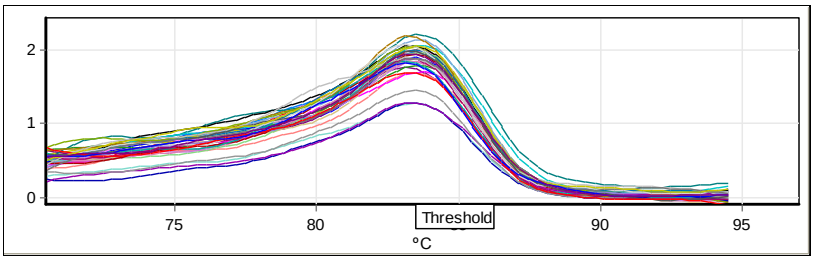

Figure (5): Beta globin dissociation curves by qPCR samples included all study groups. Melting temperature ranged from 81°C to 83°C. The photograph was taken directly from Qiagen Rotor gene Q 6000 qPCR machine.

Real-time PCR quantification of α-globin expression

There are four functional α-globin genes in a normal individual and each individual inherits two α-globin alleles

from each parent. Deletion of one or both α-gene from chromosome 16 occurs in 95% of cases, which results in the reduction of α-globin chain expression(21).

The Ct value of alphα-globin and the housekeeping gene used in the present study is shown in table (13). The Ct value was (24.75) in the control, and the Ct value was (18.65) in the patients. The fold gene expression for the control group was 1.00 and for the β-thalassemia patients, the group was (68.68).

Table (13): Fold of Alpha globin expression depending on $2^{-\Delta Ct}$method

Groups	Means Ct of α-globin	Means Ct of *B actin*	ΔCt (Means Ct of α-globin - Means Ct of *B actin*)	$2^{-\Delta Ct}$	experimental group/ Control group	The fold of gene expression
Group: Patient	18.65	15.492	3.158	0.1120333	0.1120/0.0016	68.68
Group: Control	24.75	15.490	9.26	0.0016	0.0016/0.0016	1

The Ct value of α-globin and the housekeeping gene used in the present study is shown in table (14). The 2-ΔΔCt value was (24.75) in the control, and the 2-ΔΔCt value was (18.65) in the patients. The fold gene expression for the control group was 1.00 and for the β-thalassemia patients, the group was (68.68).

Table (14): Fold of Alpha globin expression depending on 2-ΔΔCt method

groups	Means Ct of α-globin	Means Ct of *B actin*	ΔCt (Means Ct of α-globin - Means Ct of*B actin*)	Mean ΔCt Calibrator (ct α-globin -ct *B actin*	ΔΔCt	2-ΔΔCt	experimental group/ Control group	The fold of gene expression
Group: Patient	18.65	15.492	3.158	8.039	-4.881	29.466	29.466/0.428	68.68
Group: Control	24.75	15.490	9.26	8.039	1.221	0.428	0.428/0.428	1

The plot of each run was recorded including the amplification plots and dissociation curves, figures (6) and (7) show the amplification and dissociation curves for an alphα-globin gene.

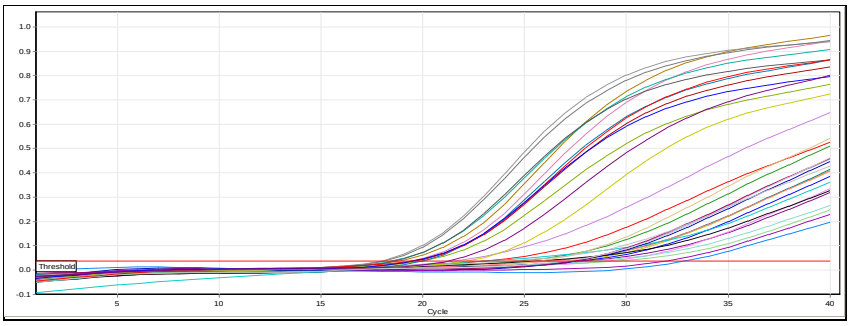

Figure (6): Alpha globin amplification plots by qPCR samples included all study groups. The photograph was taken directly from the Qiagen Rotor gene Q 6000 qPCR machine.

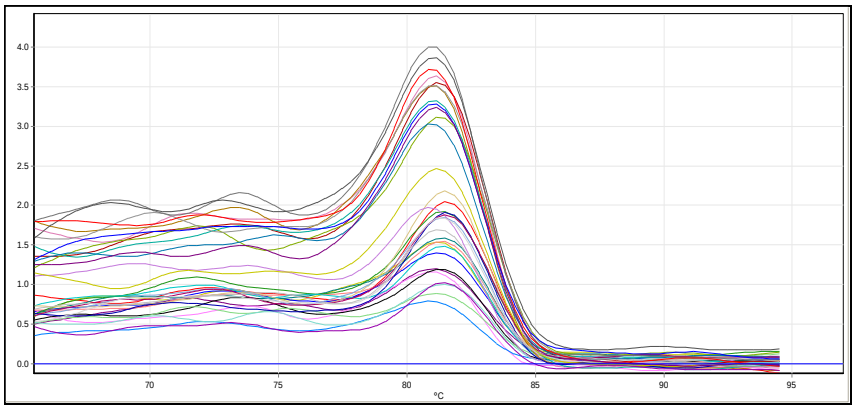

Figure (7): Alpha globin dissociation curves by qPCR samples included all study groups. Melting temperature ranged from 81°C to 84°C. The photograph was taken directly from the Qiagen Rotor gene Q 6000 qPCR machine.

Results have disagreed with Chakalova *et al.* (22) because they observed α and β globin were significantly different in β-thalassemia patients, the disagreement may be due to the difference in the gene of the USA from the Iraqi population and environmental factors, in a recent study the results appeared that there was a non-significant difference between β-thalassemia patients and control for α and β-globin.

But results were agreed with Han *et al.*, (23) and disagreed with Voon and Vadolas (24) in Australi.

Also disagreed with Ranjbaran *et al.*, (18) in Iran because they observed the β globin / α globin m RNA ratio has shown that disease severity enhanced with a decrease in this proportion. Evaluation of the correlation between AHSP gene expression and the average of the β globin / α globin expression indicated significance. Also disagreed with Mettananda *et al.*, (25) in England.

The genetic modifier is shown by a study analyzing the mRNA (7) the lower AHSP gene expression could be responsible for severe infection, Dos Santos *et al*. (26) when comparing the AHSP and α-globin gene expression in patients with thalassemia major and control by RT-PCR while AHSP gene expression was in thalassemia intermedia than in thalassemia major(27).

The reduced expression of AHSP was associated with a more severe phenotype among individuals with identical β-thalassemia and α-globin genotypes although no mutations or polymorphism in the gene could be implicated (28).

Reduced AHSP mRNA expression has been associated with clinical variability in some cases of β-thalassemia, it has been shown that α-Hb variability may also impair AHSP-αHb interaction (16).

The disease severity enhanced with a decrease in the globin/α-globin proportion. Evaluation of the correlation between AHSP gene expression and the average of the β-globin/α-globin expression ratio indicated a significant but indirect relationship in the considered groups (18).

In patients with b-thalassemia, the primary damage to the red cells and their precursors is mediated via excess α-globin chains that accumulate when the α-globin expression is reduced.

Case-control and cohort studies have demonstrated that a natural reduction in α-globin chain output, resulting from coinheritor α-thalassemia, is beneficial in patients with β-thalassemia (25).

Reduced expression of α-globin in the context of β-thalassemia can lead to considerable phenotypic improvements and α-globin is the most well-defined modifier gene known to impact the severity of β-thalassemia (24).

Comparison between control and patients for gene expression

As in table (15), the current study shows a non-significant difference in gene expression of Ct β-actin and α-globin between patients (15.49±0.02), while (15.48±0.01) in control, but the result of Ct AHSP observe a significant decrease in patients (19.92±0.02) when compared with control (20:82±0.19), also the study appears significant decrease of Ct β-globin in patients (13.73±0.11) while (15.42 ±0.12) in control.

Whereas for Ct α-globin, the results show a significant decrease in patients (18.65±0.18) compared with control (24.75±5.5).

Table (15): Comparison between control and patients in gene expression

Group	Mean ± SE			
	Ct β-actin	Ct AHSP	Ct β-globin	Ct α-globin
Control	15.48 ±0.01	20.82 ±0.14	15.42 ±0.12	24.75 ±0.50
Patients	15.49 ±0.02	19.92 ±0.02	13.73 ±0.11	18.65 ±0.18
T-test	0.049 NS	0.236 **	0.343 **	0.913 **
P-value	0.855	0.0001	0.0001	0.0001
** (P≤0.01).				

Evaluation of the concentration of alpha hemoglobin stabilizing protein (AHSP) by ELISA

Alpha hemoglobin stabilizing protein (AHSP) is an erythroid scavenger protein that quickly and reversibly binds to α subunit monomeric forms it also enhances modulation of oxidation of heme Iron and subunit folding (3).

The result of the evaluated concentrations of alpha hemoglobin stabilizing protein (AHSP) in blood serum using ELISA technique in patients with thalassemia and the control group showed non-significant differences between the two groups (Table 16) and (Figure, 8). The mean of thalassemia patients (86.79±3.44 μM^{-1}) and mean ±SE of the control group (91.11±4.49μ M^{-1})

AHSP is a small 102-residue protein expressed only in erythroid cells, which adopts a three-helix bundle.

AHSP concentration is estimated to be 0.1 mM at the end of erythropoiesis whereas that of Hb is 5 mM (29).

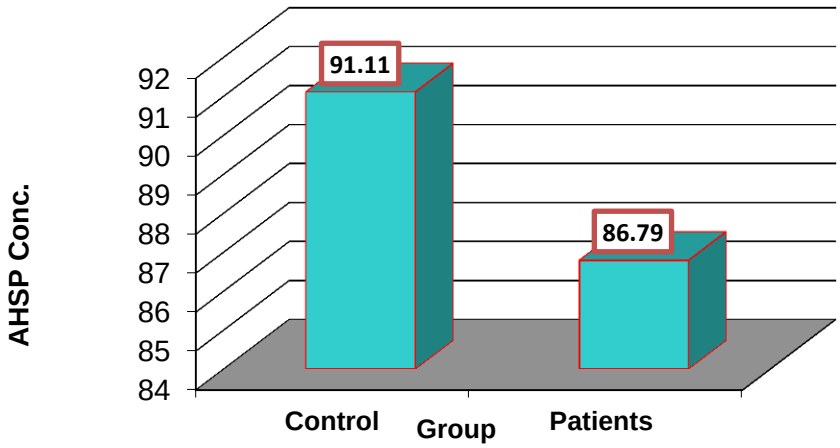

Figure (8): Comparison between control and patients in AHSP Conc.

Table (16): Comparison between control and patients in AHSP Concentration

Group	Mean ± SE of AHSP (μM^{-1})
Control	91.11 ±4.49
Patients	86.79 ±3.44
T-test	11.03 NS
P-value	0.441
NS: Non-Significant.	

The results in a table (16) and appear significant decrease in the level of AHSP in the serum of β-thalassemia major patients than in control. Ahmedy, (3) observed that the level of AHSP was high in an intermediate group of thalassemia when compared with B-thalassemia major.

AHSP helps stabilize and solubility of the α-globulin chain to avoid effective erythropoiesis by forming a stable but reversible complex with free x-globin chains (30).

AHSP performs this function by enhancing the proper folding of the aHb, inhibiting auto-oxidation of holo-aHb, as well as refolding any denatured protein (6).

Brillet *et al*. (29) also showed that a low concentration of AHSP appeared in β-thalassemia patients in their study.

Ranjbaran *et al* (18) in Iran showed a high level of AHSP in the severe and moderate group of thalassemia, but a decrease in mild thalassemia. As well Mahmoud *et al* (28) observed a low level of AHSP in β-thalassemia patients which is compatible with our observation

In conclusion, AHSP act as a disease modifier as it is an important molecule for erythropoiesis so Impaired regulation of AHSP leads to ineffective erythropoiesis and may be associated with the severity of the disease.

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