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ORIGINAL ARTICLE

EVALUATION OF FREE THYROXINE LEVEL AND BCL11A GENE POLYMORPHISM WITH BETA-THALASSEMIA

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Summary

Background: Thalassemia` is an autosomal recessive hereditary chronic hemolytic anaemia caused by a partial or total deficit in the production of β -globin chains that make up the main adult haemoglobin. Patients with the beta-thalassemia major have changes in thyroid function and result from thyroid function tests. The B-cell lymphoma /leukemia11A (BCL11A) gene is mainly located in the human chromosome 2p16.1 region; the BCL11A gene can regulate the expression of fetal haemoglobin.

The aim: analysis of the association between beta-thalassemia and the BCL11A gene polymorphism in the Iraqi patient and to evaluate the effect of beta-thalassemia on the thyroid gland through the determination of free thyroxine concentration.

Method: There were 150 participants in this study, split into two primary groups beta-thalassemia patients and healthy individuals. The result is measured using the ELISA for measurement of free thyroxine and polymerase chain reaction techniques for amplification of B-cell lymphoma /leukemia11A (BCL11A) gene polymorphism.

Results: The findings showed a substantial drop in free thyroxine levels in beta-thalassemia patient groups as compared to the control group (P 0.01). The BCL11A gene has three alleles: homozygous CC, heterozygous TC, and homozygous TT. At (431 bais pair and 280 bais pair), (431 bais pair, 280 bais pair, and 195 bais pair), and (431 bais pair, and 195 bais pair), the bands appeared, respectively. The BCL11A rs11886868 gene is affected overall, and research into the causes of thalassemia found a substantial correlation between the BCL11A (rs 11886868) T and C-alleles and thalassemia (P-value = 0.004). The fact that these people have low serum thyroxine (T4) levels highlights the importance of routine screening to assess their endocrine function.

Key words: BCL11A gene; homozygous; heterozygous; thyroid hormone; β-globin

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Introduction

A genetic condition known as beta-thalassemia causes a deficiency in the β-globin chain. At least nine different genes direct the production of globins, changes in these genes may lead to disorders of haemoglobin production, a group of conditions that causes thalassemia. The BCL11A gene is mainly located in the human chromosome 2p16.1 region; the BCL11A gene can regulate the expression of HbF (1-3).

Beta-thalassemia patients don't create enough beta protein. Two genes from each parent make up the beta-globin protein chains. The illness is referred to as "beta-thalassemia mild" or a "beta-thalassemia trait" if only one gene is faulty and causes moderate signs and symptoms in the affected person. It is known as beta-thalassemia major or Cooley's anaemia if a person has two defective genes and exhibits moderate to severe symptoms (4, 5).

There is accumulating evidence that thalassemia coexisted with other endocrine abnormalities perhaps might be the underlying causative agents for these abnormalities including thyroid dysfunctions (6, 7). However, the available evidence provides non-clear outcomes regarding the link between thalassemia and thyroid dysfunction in the light of expression of the BCL11A gene. Hence, in the present study, we aimed to highlight the genetic expression of the BCL11A gene in thalassemia patients with associated clinical thyroid outcomes.

Materials and Methods

A total of 150 participants (90 control and 60 thalassemia patients) were enrolled in the present case-control study. Samples were collected from patients at the Al-Diwaniyah Teaching Hospital and the town's thalassemia centre. The collection of samples began on 7/11/2021 and was completed on 10/5/2022. A consent form of agreement to participate in the study was collected from patient and control groups. Patients with β-thalassemia were enrolled in the study. patients were excluded from the study if they were also suffering from hypertension, alpha thalassemia, sickle cell anaemia, and iron deficiency anaemia.

Blood was withdrawn from healthy and patient groups, partly used for DNA extraction and partly used for serum analysis for thyroxine estimation. DNA extraction was conducted according to the manufacturer's instructions for the DNA extraction kit (Addbio, Sweden). Agarose gel electrophoresis is used to confirm the presence and integrity of the extracted DNA.

Sandwich-ELISA technique was used for thyroxine estimation using the kit supplied by (Sunlong biotech Co., China) as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to T4. Standards or samples are added to the appropriate Microelisa stripplate wells and combined with the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for T4 is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells containing T4 and HRP conjugated T4 antibodies will appear blue and then turn yellow after adding the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm. The OD value is proportional to the concentration of T4. You can calculate the concentration of T4 in the samples by comparing the OD of the samples to the standard curve.

Statistical Analysis

Utilizing SPSS, GraphPad Prism, and Microsoft Office Excel 2013, data were compiled, examined, and presented as mean standard deviation. Correlation analysis and ANOVA were used to calculate the level of significance. The P-value was deemed significant when it was less than 0.05.

Results

Assessing the free thyroxine level in patients with beta-thalassemia:

The findings of the current study showed that levels of fT4 were significantly lower in beta-thalassemia patient groups than in the control group (P=0.01) Table 1, Figure 1.

Table 1. Serum level of fT4 in beta-thalassemia patients compared to the control group.

Parameter	Statistics	Control group	Patient group	P-value
fT4 (pg/ml)	Mean	21.75	5.73	P<0.01
	Median	4.19	4.37	
	Standard Deviation	41.40	4.391	
	Standard Error	3.439	0.906	
	Minimum	0.912	1.99	
	Maximum	138.0	27.85	
	Range	137.09	25.86	
	Interquartile range	2.67	1.22	
	The coefficient of variation	76.61	190.37	
	Kolmogorov- Smirnova	0.000	0.000	
	Shapiro-Wilk	0.000	0.000	

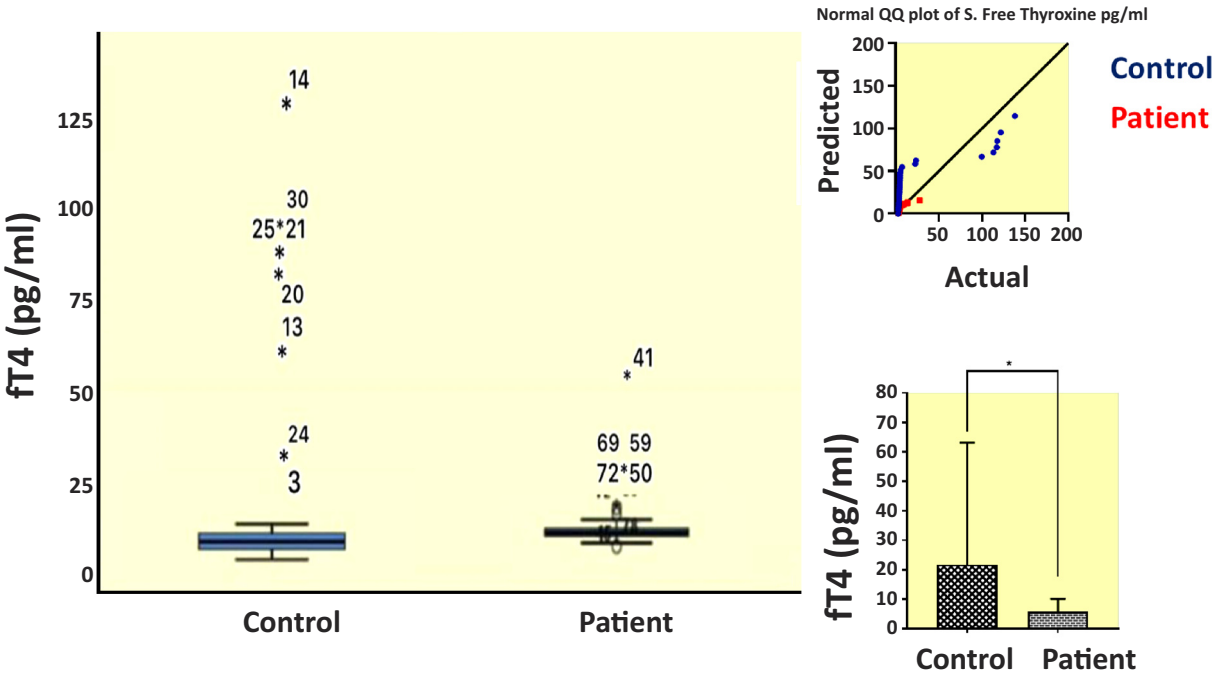

Figure 1. fT4 levels in patient groups with beta-thalassemia compared to the control group.

Association between BCL11A (rs11886868) gene polymorphism and risk of thalassemia

Table 2 displays the genotype frequencies and allele distributions of the thalassemia patients (G2) and controls (G1) groups for the T/C polymorphism of BCL11A (1). The findings showed a significant correlation between (BCL11A) T, C-alleles and risk of thalassemia illness, where X^2 is 11.06 and (P-value = 0.004< 0.05). This demonstrates a strong correlation between SNP and the risk of thalassemia. Additionally, the findings reveal that in G1, the frequencies for TT, CC, and TC were respectively 67.77%, 21.11%, and 11.11%. (Control). In G2, the frequencies for TT, CC, and TC were respectively 43.33%, 26.66%, and 30%. (Thalassemia patients). As indicated in Table 2, there was a significant correlation between G1 healthy (Control) and G2 (thalassemia patients) for the rs11886868 polymorphism of BCL11A (P-value 0.05). As a result, the TT allele was significant (P-value = 0.007 0.05). In G1, the frequency of the T allele was 132 (73.33%), that of the C allele was 48 (26.66%), and in G2, the frequencies for the T and C alleles were 70 (58.33%) and 50 (41.66%), respectively.

Table 2. BCL11A Gene polymorphism SNP distribution frequencies in patient groups with beta-thalassemia compared to the control group. Comparison between G1 & G2.

Polymorphisms BCL11A (T/C) Genotypes and Allele	Controls N=90 G1	Patients N=60 G2	X^2	P value	OR (95% CI)	P value
Genotypes						
TT	61 (67.77)	26 (43.33)			1.0 ref (1.0ref)	
CC	19 (21.11)	16 (26.66)	11.06	0.004*	0.506 (0.226-1.136)	0.096
TC	10 (11.11)	18 (30)			0.237 (0.096-0.582)	0.001*
TT	61 (67.77)	26 (43.33)			1.0ref (1.0ref)	
vs			8.83	0.003*		
CC & TC	29 (32.22)	34 (56.66)			0.364 (0.185-0.714)	
CC	19 (21.11)	16 (26.66)			1.0ref (1.0ref)	
vs			0.621	0.431		
TT & TC	71 (78.88)	44 (73.33)			0.736 (0.343-1.580)	
Alleles						
T allele	132 (73.33)	70 (58.33)			1.0ref (1.0ref)	
			7.365	0.007*		
C allele	48 (26.66)	50 (41.66)			0.509 (0.312-0.832)	

Amplification of BCL11A gene polymorphism

The BCL11A gene polymorphism (rs11886868) amplification product consists of three genotypes, the values of which bands were determined from Figures 2, 3, and 4 and those values are displayed in Table 3.

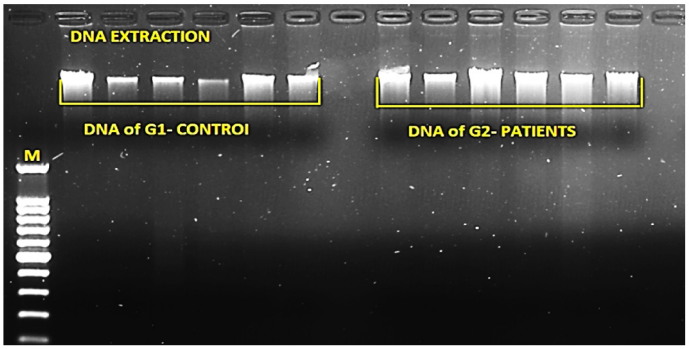

Figure 2. Genomic DNA was extracted using 1% agarose gel electrophoresis at 50 volts per cm for 45 minutes. M: DNA stairway (100-3000 bais pair). then trained with a safe, green stain and visualized under UV light.

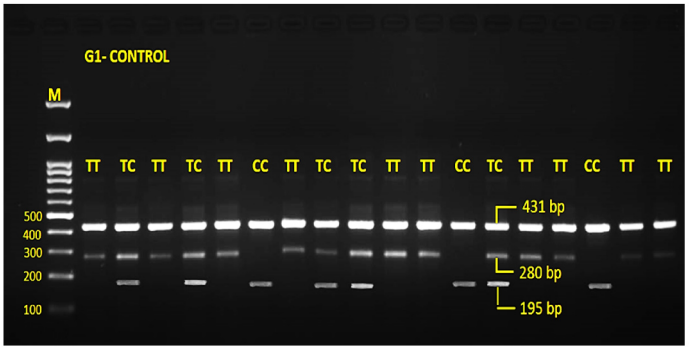

Figure 3. Employing standard PCR and gel electrophoresis to analyze the BCL11A gene polymorphism for the control group (G1). M: DNA stairway (100-3000 bais pair). PCR products were dyed using a secure dye. TT genotypes (431 and 280 bais pair), TC genotypes (431, 280, and 195 bais pair), and CC genotypes (431 and 280 bais pair) are homozygous (431 and 195 bais pair).

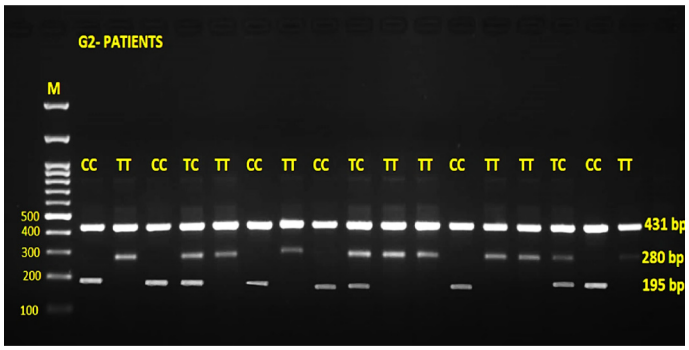

Figure 4. Using traditional PCR and gel electrophoresis to analyze BCL11A gene polymorphism in patients (G2). M: DNA stairway (100-3000 bais pair). PCR products were dyed using a secure dye. TT genotypes (431 and 280 bais pair), TC genotypes (431, 280, and 195 bais pair), and CC genotypes (431 and 280 bais pair) are homozygous (431 and 195 base pair).

Table 3. The Size of bands of BCL11A gene polymorphism.

Genotype	Number of bands	Size of bands (bais pair)
Homozygous TT	2	(431 and 280)
Heterozygous TC	3	(431, 280, and 195)
Homozygous CC	2	(431 and 195)

The BCL11A gene contains the homozygous TT, heterozygous TC, and homozygous CC alleles. The bands were visible at positions 431 bais pair and 280 bais pair, 431 bais pair, 280 bais pair, and 195 bais pair, and 431 bais pair and 195 bais pair, respectively. For controls (G1) and thalassemia patients (G2), the percentage of alleles was determined using data from these alleles and is depicted in Figures 2, 3, and 4 correspondingly.

Discussion

The present study was undertaken to analyze β-thalassemia patients for the single nucleotide polymorphism (SNP) in the BCL11A gene and to evaluate the association between this polymorphism and the severity of β-thalassemia. The results of our study exhibited downregulation of all genotypes (TT, CC, and TC) and the T allele. The results showed a significant difference in genotypic and allelic frequencies of BCL11A gene polymorphism. The frequency of TT, CC, and TC variant genotypes was found to be 43.33%, 26.66%, and 30% in the studied sample. However, the frequency of the CC genotype is 28.8 percent in the Iranian population (8). Galanello has reported the frequency of CC genotype to be 61.2 percent in Sardinians. The frequency of variant genotypes between the current observation and these observations might be due to ethnic differences (9).

Frequent blood transfusion can result in iron overload, which may lead to various complications. Most complications are caused by increased iron sedimentation in tissues like the heart and endocrine glands, resulting in heart failure, arrhythmia, hypothyroidism, and diabetes mellitus (10). Our study found reduced levels of free thyroxine in beta-thalassemia patients compared with the control group.

The commonest form of thyroid dysfunction seen in thalassemia is primary hypothyroidism. However, the frequency of hypothyroidism varies depending on the region, quality of management, and treatment protocols. It has been demonstrated that thyroid abnormalities in these patients are related to iron overload (11, 12). The precise mechanism by which iron overload causes thyroid tissue damage is not completely understood. However, it is suggested that tissue iron deposits act at the cellular level causing damage via free radical formation and lipid peroxidation resulting in mitochondrial, lysosomal, and sarcolemmal membrane damage (13, 14). Thyroid potential involvement in thalassemia could be represented as a mirror for other endocrine abnormalities (15, 16), or tissue destruction potentials (17).

The limitation of the present study is that we cannot differentiate between patients whether they are homozygotic or heterozygotic and which of the haplotypes has more predictive potential for thalassemia. Moreover, thalassemia intermedia and thalassemia minor has not been reported as a part of the study, which might give a different view regarding their potential correlation with genetic abnormalities and thyroid involvement.

Conclusion

To sum up, we concluded a close relationship between the cause of thalassemia and the BCL11A gene. Specifically, the TC genotype has been reported to be greatly involved in thalassemia. The outcome of the present study could highlight the potential use of serum thyroxine levels in the screening for the evaluation of endocrine abnormalities, alongside thalassemia disease.

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Conflict of interest

The authors declare no conflict of interest concerned in the present study.

Adherence to Ethical Standards

The study was approved by the College of Medicine, University of Al-Qadisiyah (approval letter 30/1168 on 23 March 2022).

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