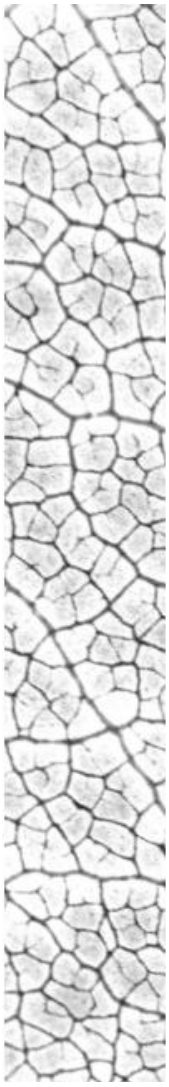

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β-globin gene (XmnI) polymorphism relation to β-thalassemia patients

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Abstract
Our study aims to the investigation of polymorphism of the β-globin gene (XmnI) in children patients with β-thalassemia in Diwaniyah province/ Iraq. the study is done by taking of the blood sample from (30) children patients with β-thalassemia at (4-10) years old, and (20) individual healthy used as control at (5-10) years old children from Al-Diwaniyah Hospital, at Diwaniyah province, Iraq. The blood samples were subjected placed in EDTA tubes and stored in the freezer until DNA extraction and make PCR-RFLP Technique by using specific primers that provided for this purpose, then the samples submitted to immigration and separation by (2) % agarose gel electrophoresis then visualized under ultra-violate Transilluminator. Our findings are showed a percentage of the XmnI-/ - (homozygous wild type) of the beta-globin gene 25/30 (83.3) %, while the percentage of the XmnI +/- (heterozygous) of the beta-globin gene was 5/30 (16.6) % in children patients with beta. Furthermore, our study showed all the children healthy group have XmnI -/ - homozygous wild type of the beta-globin gene. Finally, the most children patients with beta-thalassemia have two same polymorphism of the beta-globin gene (homozygous) at (83.3) %, while rest children patients with beta-thalassemia have two different polymorphism of the beta-globin gene (heterozygous) at (16) % furthermore the healthy group (control) have homozygous alleles of the beta-globin in normal children.

Keywords: β-globin gene, (XmnI), polymorphism, β-thalassemia

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INTRODUCTION

Beta-thalassemias are hereditary disorders related to the blood, it characterized by anomalies in the production of the hemoglobin beta chains leading to several clinical signs ranged from severe anemia to without clinical signs (Galanello and Origa, 2010; Matta et al. 2014).

Beta-thalassemia is occurring due to many types of mutations or sometimes occurs due to the deleting gene of the beta-globin on the eleventh chromosome (Cao and Galanello, 2010). The point mutations cause negative effects on the beta-globin gene including splicing, translation, and transcriptional (Karimi et al. 2014).

The β-thalassemia is occurring due to a decrease or absence of the beta-globin gene due to mutation occurrence in the gene (Nienhuis and Nathan, 2012). Production of the fetal hemoglobin at large amounts is a genetic factor of disease, with polymorphisms in other genes. Iron overload occurs due to RBC transfusions leading to an increase in iron in the many tissues including the liver, heart, and endocrine glands resulting in organs dysfunction (Farmaki et al. 2010). The chelation has a great impact in removing iron from the tissues (Fibach and Rachmilewitz, 2017).

Many of the point mutations cause Beta-thalassemias or occur due to deleting of the beta-globin gene results in a decrease of (beta+) or disappearance (beta0) production of the beta chains (Galanello and Origa, 2010). Studying the molecular basis of beta-thalassemia is giving us the ability to predicting the clinical signs from a genetic basis (Winichagoon et al 2000). Many researchers showed that increased fatal hemoglobin synthesis has a great ameliorating impact in persons who have mild form despite it is being homozygotes or heterozygotes (Dedoussis et al. 2000) (Garner et al. 2004).

This increase in fatal hemoglobin synthesis is associated with b-haplotypes that revealed the XmnI polymorphism and/or microsatellite sequences (Agouti et al. 2007). The association of the Xmn1 site with some b-globin mutations is elevated fatal hemoglobin expression (Grosso et al. 2008).

Aim of our study is the investigation of the presence of the XmnI-/ - (homozygous wild type) and XmnI-/ -

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Table 1. The used material in our study with its concentrations

The material	The concentration
DNA polymerase	I U
dNTPs	250µM
Tris-HCl	(pH 9.0) 10mM
KCl	30mM,
MgCl2	1.5mM
the stabilizer	
The tracking dye	

(heterozygous) of the beta-globin gene polymorphism in patients with beta-thalassemia.

MATERIALS AND METHODS

Blood sample collection

Blood samples were collected from (30) patients with β-thalassemia at (4-10) years old, and (20) individual healthy used as control at (5-10) years old children from Al-Diwaniyah Hospital, Diwaniyah, Iraq.

The blood samples were subjected placed in EDTA tubes and stored in the freezer until DNA extraction.

Blood DNA extraction

The Blood samples were extracted by using the Genomic Blood DNA Mini Kit (Geneaid company, Tawain), and its method was done according to kit instruction. The extracted DNA was checked for purity and the quantity using a NanoDrop. DNA kept at (-08) ºC for using it in PCR–RFLP.

PCR-RFLP Technique

PCR-RFLP technique was performed for the identification of polymorphism of the beta-globin gene -158 (C>T) XMN1 in the children patient with beta-thalassemia. blood samples and compared with healthy control children's blood samples. The technique was done according to (Said and Abdel- Salam, 2015).

RFLP PCR primer for β-globin gene (XmnI) polymorphism includes forward primer (5'-AACTGTTGCTTTATAGGATTTT-3') and Reverse primer (5'-AGGA GCTTATTGATAACTCAG AC3'), to amplified at (650) bp fragment. These primers were provided by Macrogen Company, in Korea.

The PCR master mix was prepared by using AccuPower®, while the PCR PreMix kit from Bioneer is used for doing this process according to the company directions.

The PCR premix tube contains many compounds as **Table 1**.

Preparation of the mixture is doing by adding DNA (5) µl with each primer (1.5) µl and complete the volume to (20) µl. Besides, deionized H2O at (12) µlm then the Thermocycler (Biorad company, made in the USA) was used.

Table 2. The cycles, its temperature and its periods

The cycle	Temperature	Time
The denaturation stage	(94) °C	(5) min
The annealing stage	(55) °C	(1) min
The extension stage	(72) °C	(1) min
The finishing stage	(72) °C	(10) min

The conditions which doing in the Thermocycler was included as **Table 2**.

The products are separated on the agarose gel by the electrophoresis after stained it by the ethidium bromide then visualized by the ultraviolet imager.

RFLP Step:

The RFLP step was done by using (XMN1 restriction enzyme, Biolabs, UK) restriction enzyme. Digestion products were included, the wild genotype is still undigested whereas, the heterozygous genotype gives (2) bands at (400) bp and (250) bp.

Fragments of the RFLP-PCR were migrated by using the electrophoresis that contains the stain and visualized at the ultraviolet device.

RESULTS

Our study found according to the table (1) and (2), percentage of the XmnI-/ - (homozygous wild type) of the beta-globin gene 25/30 (83.3) % while the percentage of the XmnI+/- (heterozygous) of the beta-globin gene was 5/30 (16.6) % in children patients with beta-thalassemia as **Table 3** and **Table 4**.

Based on our study, all the children healthy group have XmnI-/ - homozygous wild type of the beta-globin gene as shown in **Table 5**.

As below, the image showed that lane (1, 2, and 5) XmnI+/- (heterozygous genotype) are digested by restriction enzyme into (400) bp and (250) bp bands while the other Lanes are XmnI-/ - (homozygous wild type) were showed undigested (650) bp bands as **Fig. 1**.

DISCUSSION

Thalassemia is a common genetic disorder associated with several clinical symptoms included hemolytic anemia (Zandiankh et al. 2006; Farshdousti et al. 2011). Positive XmnI gene polymorphism was the main phenotype factor in the patients with beta-thalassemia for testing the relationship between genotypic and phenotypic of the XmnI polymorphism in the beta-thalassemia (Boudrahem-Addour et al. 2009; Neishabury et al. 2010).

According to our results, our results showed a percentage of the XmnI-/ - (homozygous wild type) of the beta-globin gene 25/30 (83.3) %, while the percentage of the XmnI+/- (heterozygous) of the beta-globin gene was 5/30 (16.6) % in children patients with beta. While

Table 3. The number of the sample and its results that related with XmnI-/- (homozygous wild type) and XmnI+/- (heterozygous) of the beta-globin gene in children patient group

Sample N.	XmnI-/ - (homozygous wild type)	XmnI+/- (heterozygous)
1	Positive	
2	Positive	
3	Positive	
4		Positive
5	Positive	
6	Positive	
7		Positive
8	Positive	
9	Positive	
10	Positive	
11	Positive	
12		Positive
13	Positive	
14	Positive	
15	Positive	
16	Positive	
17	Positive	
18		Positive
19	Positive	
20	Positive	
21	Positive	
22	Positive	
23	Positive	
24		Positive
25	Positive	
26	Positive	
27	Positive	
28	Positive	
29	Positive	
30	Positive	

Table 4. number and percentage of the polymorphism of the beta-globin in children patients

	XmnI-/ - (homozygous wild type)		XmnI+/- (heterozygous)	
Total	Number	Percentage	Number	Percentage
30	25	83.3	5	16.6

Table 5. The number of the sample and its results that related with XmnI-/- (homozygous wild type) and XmnI+/- (heterozygous) in the patients group in the control group

Sample N.	XmnI-/ - (homozygous wild type)	XmnI+/- (heterozygous)
1	Positive	
2	Positive	
3	Positive	
4	Positive	
5	Positive	
6	Positive	
7	Positive	
8	Positive	
9	Positive	
10	Positive	
11	Positive	
12	Positive	
13	Positive	
14	Positive	
15	Positive	
16	Positive	
17	Positive	
18	Positive	
19	Positive	
20	Positive	

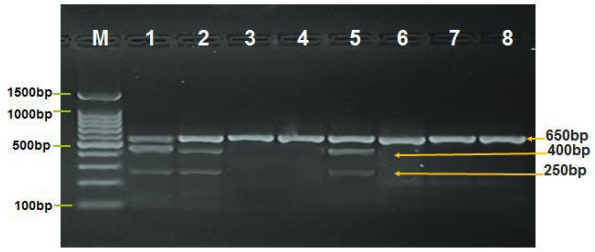

Fig. 1. The band on the electrophoresis in the RFLP-PCR of the beta-globin gene -158 (C >T) XMN1 polymorphism in the children patients with beta-thalassemia group and control group by using XmnI restriction enzyme in 2% agarose. M indicates to the marker (1500-100bp), lane (1, 2, and 5) XmnI+/- (heterozygous genotype) that show digested by restriction enzyme into 400bp and 250bp bands. Other Lanes are XmnI-/ - (homozygous wild type) were showed undigested 650bp bands

our study showed all the children healthy group have XmnI-/ - homozygous wild type of the beta-globin gene.

The marriage of two heterozygous individuals for β-thalassemia (thalassemia minor patients), there is (25) % chance of homozygous patients, 50% chance of heterozygous birth carrying the disease gene, and 25% chance of the birth of a healthy homozygous individual (Lo, 2005; Mavrou et al. 2007; Galanello and Origa, 2010).

(19) are (316) samples for examination of the prenatal diagnosis, (56.8%) of the cases have a least single mutated gene of beta-thalassemia, and the study revealed which IVS II-1 (G > A) and CD 36-37 (- T) polymorphisms are most common at 10.1% and 13.9% respectively (Dehghanifard et al. 2013). Few numbers of reports showed that the relationship between phenotypic and genotypic of XmnI gene polymorphism in beta-thalassemia (Neishabury et al. 2010; Bahadir 2012).

There are two studies showed that studied the prelateship between the phenotypic and genotypic homogenous and heterogenous of the beta-globin in the patients with b-thalassemia at (9) % and (4) % receptively (Kaddah et, al. 2009; Tantawy et al. 2012).

The percentage of the polymorphism of the beta-globin gene in patients with beta-thalassemia is varying, and that is normal results because that depended on the married individuals (Aessopos et al. 2005; Giardine et al. 2007).

CONCLUSION

In conclusion, molecular detection of the genetic factor in the first stages of the childhood will help us to the identification of the disease in the future also, prevent or decrease the clinical signs, and give us new early diagnosis for early treatment. The studies that related to the genetic markers will be leading to predicting the presence of the disease.

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