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Original article

Biochemical and molecular analysis of the beta-globin gene and LCR region on Saudi β-thalassemia patients

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ABSTRACT

Introduction: Beta-thalassemias are a group of inherited blood disorders caused by reduced or absent synthesis of beta chain of hemoglobin resulting in variable phenotypes ranging from clinically asymptomatic individuals to severe anemia symptoms. The objective of this study is to screen for the whole beta gene globulin and the LCR region and its clinical relevance in β-Thalassemia patients.
Methods: In this study, we collected 140 blood patients' samples with beta-thalassemia from different areas of Saudi Arabia. DNA was then extracted then the molecular scanning for the whole β-globin gene and the Locus control region (β-LCR) for patients' samples, was run using PCR.
Results: Sixty one mutations found in this study, including 22 new mutations not recorded in the database before. These deletions including: (*C-1960-1961 ca/-- del in hbb5) and (*c-519C<T homo, *c-390C<T homo in hbb6) were the highest among beta-thalassemia in the study, which indicates a strong sign of injury associated with the disease. Meanwhile, There are other mutations found most common among patients and was linked with the severity of clinical symptoms including: (c-1960-1961 ca/-- del in hbb5), (c-519C<T homo, c-390C<T homo, c-160 G<A het in hbb6), (c.315+282 G<A het, c.316-225G<A het, c.315+342 G > A het in hbb9). Interestingly, the highest percentage in gene deletion occurred in exon 03A by ~33% of the samples, while the highest percentage in gene addition of the gene occurred in exon 03B by ~25%.
Conclusion: This study was unique to show several new mutations that would help in diagnosis and treatment. These results should be taken further to set up better management strategies to improve outcomes.

1. Introduction

Thalassemia is an inherited and heterogeneous genetic disorder results from a decreased synthesis of alpha or beta chains of hemoglobin (Hb). Hemoglobin serves as an oxygen-carrying component on RBCs. If the body does not manufacture enough of one or the other of these two proteins, the RBCs do not form correctly and cannot carry sufficient oxygen. It is caused by either a genetic mutation or a deletion of certain key gene fragments (Stamatoyannopoulos, 2001; Weatherall and Clegg, 2001; Weatherall and Clegg, 2001; Weatherall and Clegg, 1981; Webster and Lammi, 1994).

Beta Thalassemia is a hematological genetic disease caused by inability to produce beta globulin. Alpha thalassemia is prevalent in Asian and African populations while beta-thalassemia is more prevalent in the Mediterranean population, although it is relatively common in Southeast Asia and Africa too. β-Thalassemia disease is a major health problem in many countries around the world. This disease was found in the KSA (Mir et al., 2020 Jan; Al-Suliman, 2006; Al-Sultan et al., 2011; Al-Ali et al., 2005; El-Hazmi, 1982; El-Hazmi and Warsy, 1998; El-Hazmi and Warsy, 1999; El-Hazmi and al-Swailem, 1995; Alsaeed et al., 2018), GCC and Middle East (Addour et al., 2011; Al-Allawi et al., 2006; Bircan et al., 1993; Bolaman et al., 2001; Darwish et al., 2005; Derakhshandeh-Peykar et al., 2007; Afrasiabi et al., 2011) and many other countries (Abdullah et al., 1996; Agarwal et al., 2000; Hossain et al., 2017 May 18; Cao et al., 1989; Choudhry, 2018 May; Bandyopadhyay et al., 1999; George, 1998; Hanafi et al., 2014 Sep 5; Georgiou

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Table 1
Characteristics of the patients.

Number of patients	140 (80 females and 60 males); Age: 65.1 ± 3.2 Mean ± SD
Period of treatment	About 13 months recruited from KKJH
Inclusion Criteria	Age range 10–70 years AND Symptomatic [POS by PCR]. AND Written informed consent AND Excellent organ function: Renal – Pulmonary- Cardiac: No evidence of congestive heart failure, symptoms of coronary artery disease, myocardial infarction; CNS: No history of cerebrovascular accident.

et al., 2003). Therefore, β-Thalassemia is observed widely in the red sea as well as the eastern region (Table 1).

β-Thalassemia is characterized by chronic haemolysis, recurrent vasoconstriction, rapid infection, failure of various organs in the body, inflammation, stroke, acute chest pain, anaemia and jaundice. In Saudi Arabia, the β-Thalassemia was first identified in the eastern region in Al-Ali and El-Hazmi back to early 1980s, which led to the initiation of multiple studies at the regional and national level to determine the clinical characteristics and gene replication of β-Thalassemia in different regions of Saudi Arabia. Locally there are more than 1000 cases and about 50.000 carriers in the last 10 years. Internationally, every year more than 100.000 newborn die from β-Thalassemia.

Molecular techniques remain the best assays for screening and confirmation the β-Thalassemia existence and complication (Abd-Elsalam, 2003; Chang et al., 1995; Chia-Cheng and Shee-Uan Chen; Shin-Yu Lin; Mei-Ya Fang; Li-Jung Chang; Yi-Yi Tsai; Li-Ting Lin; Yu-Shih Yang; Chien-Nan Lee and Yi-Ning Su, , 2010; Fakher et al., 2007; Kazazian and Boehm, 1988; Patel et al., 2008; Pavlov et al., 2004; Mishra et al., 2012; Thokar, 2010; Singh and Kumar, 2001).

The aims of this study are to screen for the whole beta gene globulin and the LCR region and its clinical relevance in β-Thalassemia.

2. Materials and methods

2.1. Patients

We collected blood samples from 140 Saudi β-Thalassemia patients selected randomly from blood diseases clinic at King Khalid University Hospital (KKUH, Riyadh, KSA) from different regions of Saudi Arabia between Jan2017-Jan2020. Thirty healthy normal controls were also recruited to this study. Hematological and biochemical measurements and history of each patient were investigated. The study protocol respected the most recent Declaration of Helsinki, written informed consent and Research Ethics Committee approval were obtained from all cases (see Table 1).

2.2. Samples

10 ml of venous blood was withdrawn from each patient and distributed to two tubes (each containing 5 ml) of ethylenediamine tetra acetic acid (EDTA) and kept at 4C for later use.

2.3. Extraction of DNA

DNA was extracted using a Qiagen gel purification kit, according to the manufacturer's instructions. DNA concentration was measured by the Spectrophotometer 1000 Nanodrop at 260 nm.

2.4. Primers & PCR

Primers were designed, requested and obtained through the Oligo ordering online. PCR primers were used (Table 2). Thirty-five cycles of PCR, with denaturation at 94 °C for 30 s, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min, were performed on a programmed-temperature system (Hybaid Omni-Gene; Midwest Scientific, Missouri, United States). After PCR amplification, 10 μl of the PCR products were mixed with 2 μl DNA loading buffer and electrophoresed on a 2% agarose gel containing 0.2 μg/ml ethidium bromide in 0.5X TBE buffer. A DNA ladder was also run in parallel. The amplified gel was visualized and photographed under UV light (Bio-Rad Gel Doc 2000 Imaging System).

Table 2, shows the primers for β-Thalassemia of HBB gene
Table 3, shows the primers for LCR used for HBB gene
There are many techniques in this study were used including:

1. PCR for beta HBB and LCR in β-Thalassemia patients.
2. Nucleotide Sequencing using Applied Biosystems.
3. Multiplex Ligation-dependent Probe Amplification.

2.5. Statistical analysis

The data obtained was subjected to a statistical analysis using Window Excel and SPSS v17 statistical tools. ANOVAs tests for mul-

Table 2
These primers were designed and used in this study to multiply the beta-globin gene.

primer	Primer seq 5′ – 3′	Product size (b.p)
HBB-1F	GTATGCCTGGGCTTTTGATG	482
HBB-1R	CCCTGTTTCACATCCCTGAT	
HBB −2F	GCATTTTCTTTGACCCAGGA	527
HBB −2R	ACCTGTCTCAACCCTCATCA	
HBB −3F	CCAAACACTTTCTGCGTGTG	494
HBB −3R	GCAGAGTTCTGCTTCCTGCT	
HBB −4F	TGAGACCCTACGCTGACCTC	458
HBB −4R	CAATGGGGTAATCAGTGGTG	
HBB −5F	TTTTGTTCCCCCAGACACTC	471
HBB −5R	TCTTCCTGCGTCTCCAGAAT	
HBB −6F	TTTTTCTTTTCTTACCAGAAGGTTTT	470
HBB −6R	TGCTCCTGGGAGTAGATTGG	
HBB −7F	ACTCCTAAGCCAGTGCCAGA	490
HBB −7R	CAGATCCCCAAAGGACTCAA	
HBB −8F	GGCACTGACTCTCTCTGCCTA	496
HBB −8R	AAAAATTGCGGAGAAGAAAAA	
HBB −9F	TGTTTTCTTTTGTTTAATTCTTGCTTT	486
HBB −9R	TTGCTATTGCCTTAACCCAGA	
HBB −10F	TTCAGGGCAATAATGATACAATG	500
HBB −0R	GATGCTCAAGGCCCTTCATA	
HBB −11F	TTCCTTTGTTCCCTAAGTCCAA	489
HBB-11R	GGAACACTTCAGGGGAAAGG	
HBB −12F	TGCATCTCTCAGCCTTGACT	600
HBB-12R	CTTGAGACTCATATTTTATTTCCAGA	

Table 3
These primers were designed and used in this study to multiply the LCR region.

primer	Primer seq 5′ – 3′	Product size (b.p)
LCR-1F	CCTGCAAGTTATCTGGTCAC	445
LCR-1R	CTTAGGGGCTTATTTTATTTTGT	
LCR-2F	CAGGGCAGATGGCAAAAA	460
LCR-2R	CTGACCCCGTATGTGAGCA	
LCR-3F	ATGGGGCAATGAAGCAAAGGAA	595
LCR-3R	ACCCATACATAGGAAGCCCATAGC	
LCR-4F	GCAAACACAGCAAACACAACGAC	442
LCR-4R	CAGGGCAAGCCATCTCATAGC	
LCR-5F	GGCCCCTTCCCCACACTATC	544
LCR-5R	ATGGCAGAGGCAGAGGACAGGTTG	
LCR-6F	TTCCCAAAACCTAATAAGTAAC	520
LCR-6R	CCTCAGCCCTCCCTCTAA	
LCR-7F	TGCCCTGGCCCACAAGTATC	539
LCR-7R	TCAGGGGAAAGGTGGTATCTCTAA	

tiple comparisons and significant analysis ($p<0.05$) were carried out.

3. Results

3.1. Part-1:Results of nucleotide screening of the beta globin in β-Thalassemia patients

In order to fully understand and diagnose β-Thalassemia, there many techniques, however, we made a full screening for nucleotide sequence plus 6 LCR regions by using PCR to indentify all existing mutations, later, we used a revlusionalized techniques name as Multiplex ligation dependent probe amplification) MLPA). We identified two changes; those in β-Thalassemia patients not in normal controls, called (diseased changes). While the others found in normal controls, called (polymorphic changes), including substitution, deletion and insertion. After searching in international known database, we divided these changes as follows:

1. We also identified several changes that had no Reference Registration Numbers in international Database
   15 changes in the β-Thalassemia patients (as shown in Table 4)
   7 changes in normal people (as shown in Table 5)
2. Additionally, we indentified several changes and had Reference Registration Numbers in international Database

   24 changes in the β-Thalassemia patients (as shown in Table 6)
   15 changes in normal people (as shown in Table 7)

We, then, studied the relationship between clinical signs and disease severity and changes in β-Thalassemia. We found low RBC count, while MCH was 55FL, Hb level was 6.1 g/l and significant elevation of Hb-F and HbA2.

A significant relationship was found in segment no-3 between *C-1271 G<A het and high Bilirubin, as shown in Table 8.

A significant relationship was found in segment no-4 between c-390C<T homo and WBC count and Ferritin, as shown in Table 9.

A significant relationship was found in segment no-6 between *C- 1039C<G homo & *c-390C<T homo and LDH and Bilirubin, as shown in Table 10.

A significant relationship was found in segment no-6 between *c-390C<T homo and splenoectomy, as shown in Table11.

Moreover, We registered 83 new changes in β-Thalassemia patients (diseased state), and 3 in the control group.

3.2. Part-2:Results of nucleotide screening of the beta LCR region in β-Thalassemia patients

LCR region of β-Thalassemia was identified and propagated using the primers designed in Table (Weatherall and Clegg, 2001). The analysis of the nucleotide sequencing for the entire LCR segment was also determined. It is important to note that there were many changes in LCR region as follows below:

- The first fragment LCR-HS1 shows 15 changes
- The second fragment LCR-HS2 shows 5 changes
- The third fragment LCR-HS3 shows 29 changes

Table 4
New changes obtained from β-thalassemia patients, had no reference number.

Segment of gene	Change	Mutation	Reference Number	%
HBB/F1	c*-2049 T<-	Del	No-Ref Number	3.5%
HBB/F2	*c.-1440–1438 TTT /-	Del	No-Ref Number	10.0%
	*c.-1442–1436 ATTTTG/-	Del	No-Ref Number	10.0%
HBB/F3	*c-1271 G<A	Het	No-Ref Number	16.4%
HBB/F4	*c-870C<T	Het	No-Ref Number	3.5%
HBB/F 5	*c −559 G<A	Het	No-Ref Number	3.5%
	*c −536 G<A	Het	No-Ref Number	3.5%
	*c −533 G<A	Het	No-Ref Number	3.5%
	*c −523 G<A	Het	No-Ref Number	3.5%
	*c-668 G<C	Het	No-Ref Number	3.5%
HBB/F 6	*c-160 G<A	Het	No-Ref Number	62.0%
	*c-195 G<A	Het	No-Ref Number	10.0%
	*c-192 G<A	Het	No-Ref Number	16.4%
HBB/F8	c.315+38 T<C	Homo	No-Ref Number	3.5%
HBB/F9	c.316–247 T<G	Het	No-Ref Number	26.4%

Table 5
New changes obtained from normal controls, had no reference number.

Segment of gene	change	Mutation	Reference No	%
HBB/F9	c.315+282G<A	Het	No-Ref Number	100.0%
	c.316-225G<A	Het	No-Ref Number	100.0%
	c.315+342 G<A	Het	No-Ref Number	100.0%
HBB/F12	*C+828 G<A	Homo	No-Ref Number	36.4%
	*C+828 G<A	Het		23.5%
	*C+786C<G	Homo	No-Ref Number	50.0%
	*C+786C<G	Het		26.4%

Table 6
New changes obtained from β-thalassemia patients, registered with reference number.

Segment of gene	Change	Mutation	Reference No	%
HBB/F1	c*-2121C<G	Het	Rs# 60025376	13.5%
HBB/F2	*C-1934C<A	Het	Rs# 777867	10.0%
	*c.-1578C<T	Het	Rs# 58919412	10.0%
HBB/F3	*c – 905–910 -/tttta	Ins	Rs# 59124155	6.4%
	*c-1090-1130 atttt/-	Del	Rs# 61168339	3.5%
HBB/F4	*c-1119 G<A	Homo	Rs# 1003586	13.5%
	*C- 1039C<G	Homo	Rs# 16911905	33.5%
HBB/F 5	*c.-601 T<C	Homo	Rs# 35755129	30%
	*c.-601 T<C	Het		18.5%
	*c.-753 T<C	Homo	Rs# 11036364	30.5%
	*c.-753 T<C	Het		3.5%
	*C-1980–1981 –/tt	Ins	Rs# 67721287	3.5%
	*C −571–572 –/TT	Ins	Rs# 67721287	3.5%
	*C −518C<T	Het	Rs# 10742584	3.5%
HBB/F 7	C. (17_18) CT/–	Del	Rs# 63750729c/- : Rs# CD890151 T/-	3.5%
	c.92+5 G<C	Homo	Rs# CS820004	6.4%
	C.92+6 T<C	Het	Rs# 35724775	6.4%
	C.118 CAG<TAG C<T Q40end	Het	Rs# CM810001	16.4%
HBB/F8	C.315+81 C<T	Het	Rs# 7946748	25.0%
	C.315+1 G<A	Homo	CS870042	16.4%
	315+26 T<G	Homo	Rs# TMP_ESP_11_5247781	16.4%
HBB/F11	*C+233 G<C	Het	Rs# 12788013	33.5%
	*c.+472 A<T	Het	Rs# 10837631	20.0%
	*c.+472 A<T	Homo		10.0%

Table 7
New changes obtained from normal controls, registered with reference number.

Segment of gene	Change	Mutation	Reference No	%
HBB/F2	*C-1917C<T	Homo	Rs# 7936823	70%
HBB/F 5	*C-1960–1961 ca/–	Del	Rs# 201615432	100.0%
HBB/F 6	*c-519C<T	Homo	Rs# 10742584	100.0%
	*c-390C<T	Homo	Rs# 10742583	80.5%
	*c-390C<T	Het		9.5%
HBB/F 7	c.9 t<c CAT<CAC T<C H3H	Homo	Rs# 121909815	90.0%
HBB/F8	c.315+16 G<C	Homo	Rs# 10768683	85.0%
	C.315+74 T<G	Homo	Rs# 7480526	60.0%
HBB/F10	C.316–185C<T	Het	Rs# 1609812	26.4%
	C.316–185C<T	Homo		60.0%
	C.396 CAG<CAA G<A Q132Q	Het	Rs# 34188626	96.4%
HBB/F11	*C+316 A<C	Homo	Rs# 7110263	70.0%
	*C+316 A<C	Het		23.5%
HBB/F12	*C+625 T<G	Homo	Rs# 78928216	36.4%
	*C+625 T<G	Het		23.5%

Table 8
Relationship between this change (*C-1271 G<A) of β-Thalassemia patients and their Lab investigations' results.

	Mutation +ve	Mutation –ve	P value
WBCs	14.1 ± 4.1	13.3 ± 1.5	0.85
HB%	91.7 ± 4.7	84.01 ± 2.2	0.16
LDH	245 ± 224	243 ± 22.5	0.07
Bilirubin	62.1 ± 6.8	30.1 ± 4.9	<0.01*
HBA2	2.71 ± 0.25	3.70 ± 0.26	0.12
HBF	0.72 ± 0.09	3.9 ± 1.3	0.31
Serum Ferritin	876 ± 620	1589 ± 635	0.64

Table 10
Relationship between this change (*C- 1039C<G ho & *c-390C<T homo) of β-Thalassemia patients and their Lab investigations' results.

	Mutation +ve	Mutation −ve	P value
WBCs	13.5 ± 1.6	13.2 ± 2.7	0.92
HB%	84.5 ± 2.3	87.6 ± 4.3	0.51
LDH	229 ± 23	419 ± 142	<0.04*
Bilirubin	29.8 ± 4.5	52.5 ± 2.2	<0.04*
HBA2	3.6 ± 0.3	3.3 ± 0.3	0.58
HBF	3.6 ± 1.4	2.2 ± 1.2	0.59
Serum Ferritin	1314 ± 539	1982 ± 157	0.61

Table 9
Relationship between this change (*C- 1039C<G homo) of β-Thalassemia patients and their Lab investigations' results.

	Mutation +ve Homo	Mutation +ve Het	Mutation –ve	P value
WBCs	7.8 ± 1.6	6.6 ± 2.3	15.1 ± 1.4	<0.05*
HB%	96 ± 4	96 ± 5	82.6 ± 2.1	0.01
LDH	245 ± 37	232 ± 96	287 ± 50	0.91
Bilirubin	28. 6 ± 12.7	13.5 ± 7.5	38.8 ± 5.5	0.32
HBA2	3.97 ± 0.52	3.3 ± 0.6	3.8 ± 0.28	0.79
HBF	4.3 ± 3.6	12.4 ± 11.5	2.3 ± 0.7	0.03*
Serum Ferritin	5316 ± 227	6185 ± 264	427 ± 264	< 0.001**

Table 11
Relationship between splenectomy and several mutations in β-Thalassemia patients.

P	No Splenectomy (N = 104)	Splenectomy (N = 36)	Mutation
0.25	12 (12.5)	0 (0)	Fragment 1 2196C<T
0.91	60 (62.5)	24 (66.66)	Fragment 2 1917C<T
0.93	18 (18.75)	6(16.66)	Fragment 3 1271 G<A
0.51	24 (25)	6 (16.66)	Fragment 4C<G 1039
0.09	72 (75)	36 (100)	Fragment 5 1960–1961 CA/-
<0.01*	66 (68.75)	36 (100)	Fragment 6 390C<T
0.33	60 (62.5)	30 (83.33)	Fragment 7 9C<T
0.35	84 (87.5)	24 (66.66)	Fragment 10 396 G<A
0.35	84 (87.5)	24 (66.66)	Fragment 11 316 A<C
0.27	54 (56.25)	12 (33.33)	Fragment 12 625 T<G

Table 12
New changes obtained in 7th fragment of the LCR region of β-Thalassemia patients.

%	Mutation	Change	No	segment β-LCR7
4	Homo	64081 A<T	1	F.β-LCR7
98	Ins	63918–63919 -<C	2	(start.63586 – end.64125)
8	Homo	63843 G<C	3	flanking sequences enhancer
5	Het	63843 G<C	4	
13.5	Homo	63925C<A	5	
5	Het	63925C<A	6	
0.7	Homo	63923 G<A	7	

Table 13
Table12: Deletion of a particular gene distributed between both sexes.

Location of the gene	Males N = 33 (%)	Females N = 41 (%)	Chi square	P
Significant ratio for promoter	29 (87.9)	34 (82.9)	0.35	0.55
Significant ratio for Exon 1 A	32 (96.9)	39 (95.1)	0.16	0.69
Significant ratio for Intron 1	25 (75.8)	37 (90.2)	2.28	0.09
Significant ratio for Intron 2	31 (93.9)	36 (87.8)	0.81	0.37
Significant ratio for Exon 3A	18 (54.5)	31 (75.6)	3.64	0.04*
Significant ratio for Exon 3B	31 (93.9)	4 (9.8)	0.62	0.43
Significant ratio for HBB1	29 (87.9)	39 (95.1)	1.29	0.26
Significant ratio for HBB2	32 (96.9)	41 (100)	1.26	0.26

- The fifth LCR-HS5 region shows 18 changes
- The sixth fragment LCR-HS6 shows 9 changes
- The seventh LCR-HS7 fragment shows 7 changes (most common) Table12

3.3. Part-3: Results of the MLDA amplification

In this study, we examined 74 β-Thalassemia patients to indentify the presence of mutations, we therefore used 8 combinations to cover most areas in the beta gene as follows: exon 01A, intron 0, intron 02, exon 03A, exon 03, HBB1, HBB2 and HBB promoter. They are the major control regions in the gene. It is interesting to notice that addition and deletions are variables from region to another (Table13).

A statistics program was used, after putting the MLPA results, statistical significance was found in deletion of exon 3A in both sexes, this may be due to high number of female participants in this study

4. Discussion

El-hazmi's group found that the central and western regions of the KSA have considerable cases equal to those in the east and north. It was found that the most common mutation in Saudi β-Thalassemia patients is: C93-21 GLA, followed by the Q40X GLS (El-Hazmi and Warsy, 1998; El-Hazmi and Warsy, 1999; El-Hazmi and al-Swailem, 1995). Alsulimani's group showed that Asians mutation interferes with the local Saudi mutation namely: IVS-110 and IVSII (Al-Suliman, 2006). Alsultan et al found that 196 Saudi individuals, who had blood transfusion, from eastern region of KSA, had 14 mutations: 164 had homozygous mutation and 32 have heterozygous mutation. While those who had low blood transfusion in their life, had heterozygous mutation (Al-Sultan et al., 2011)

LCR experiments was also run using PCR technique, then analyzed the sequences, and evaluated the results using the seqMan program, to identify mutations, renamed, and documented in the database. Multiplex ligation-dependent probe amplification Procedure (MLPA) is based on the cloning of several sites in the genome using multiple primers in the PCR reaction, called Multiplex PCR, It can be operated at more than 50 targets along the DNA and this method is a sensitive technique to any mutation.

It was found that there is a wide spectrum of mutations upto 61 changes that were identified in all parts of the beta-globin gene in this study, which included homogeneous and heterogeneous changes, these results were in agreement with Mishra et al (Mishra et al., 2012).

In total, there were two types of changes, pathological changes found in patient samples only and natural changes found in both patient and control samples that did not show any symptoms of the β-Thalassemia disease. 22 natural changes were recorded in this study, 7 of which were not previously recorded in the global database including those [C+828 G<A homo* ، C+828 G<A het* ، *C+786C<G homo، *C+786C<G het) (*c-2049T<- del، *c.-1440–1438 TTT /- del ، *c.-1442-1436 ATTTG/- del ، *C-1271 G<A het ، *C-870C<T het ، *C -559 G<A het، *C -536 G<A het ،*C -533 G<A het ،*C -523 G<A het ، *c-668 G<C het ، *c-160 G<A het ،*c-195 G<A het، *c-192 G<A het ، c.315+38 T<C homo، c. 316-247T<G het]

After searching the global database in the human genome (http://www.ensembl.org/index.html), 22 new changes in the beta-globin gene were identified in this study, for which no reference number was found, a registration number will be referenced for later genetic studies.

From what has been noticed that this change (c.9t<c<cAC T<C H3H homo) was found by 90% of samples of β-Thalassemia patients in this study, however it was also found that the most severe cases of β-Thalassemia were found within 10%, and the clinical symptoms of these patients had blood transfusions 12–17 times / 12 months, as well as hospital admission from 12 to 17 times / 12 months. Since the severity of beta thalassemia is dependent on the severity of the clinical symptoms of β-Thalassemia patients, samples were categorized according to the number of blood transfusions and the amount of blood transfused, as well as the number of admission and splenoectomy.

It was found that the common changes between samples of β-Thalassemia patients were normal changes (c.315+282G<A het, c.316-225G<A het, c.315+342 G<A het) in HBB9 that were not previously recorded, in addition to changes that were recorded previously (* C-1960–1961 ca / - del) in HBB5 and * c-519C<T homo), * c-390C<T homo) in HBB6. The most important is the pathological change that was recorded in this study and was not previously recorded (* c-160 G<A het) in HBB6.

Statistical analysis showed that there was a correlation at the splitting level and this correlation was of variable. In the second segment of the beta-globin gene, two previously recorded changes were found (* C-1934C<A het, * c.-1578C<T het) and the change recorded in this study was (* c.-1440-1438 TTT / - del). The three changes also showed a correlation with a 99% confidence degree among them at the same segment.

There was a positive correlation between the change reported in this study * C-1271 G<A het in the third segment of the beta-globin gene and high Bilirubin level, as this accompanied by 7 times blood transfusion during the last 12 months, plus using Hydroxurea and splenoectomy

Minor clinical symptoms were observed in a patient with this change (* c-2049 T <- del). There was an elevation of Bilirubin and LDH and high WBC and during the last 12 months, no blood transfusion or admission was reported, splenoectomy and hydroxyurea was not taken. It was found that this change (C. (17_18) CT / - del) found in the seventh piece of the beta-globin gene

Those patients who had this change (* C-870C<T het) had high LDH and high Bilirubin during the last 12 months.

Very mild symptoms were observed in the patient who had the change (* c-668 G<C het) in the fifth segment of the gene including Bilirubin and LDH and WBC.

This change (* c-390C<T homo) in the sixth piece of the beta-globin gene found to be 62% of the patients under study, was correlated with high LDH and high Bilirubin, plus they had blood transfused 4 times and treated with hydroxyurea.

The two variations recorded in this study in the sixth piece of the beta-globin gene (c-195 G<A het), (c-192 G<A het) are correlated with each other directly, in both patients had blood transfusion more than 6 times, splenctomy and under Hydroxyurea

There was a correlation between the change (C- 1039C<G homo) in the fourth segment of the beta-globin gene and high WBCs. it was also found that Serum Ferritin is associated with this change. This patient had Blood transfused for more than 12 times splenoectomy

In 14% of patients with beta thalassemia of this study who had deletion [C. (Bircan et al., 1993; Bolaman et al., 2001) CT / homo del], high Bilirubin, high WBC and low LDH (El-Hazmi and Warsy, 1999; El-Hazmi and al-Swailem, 1995)].

The deletion change (C-1960-1961 ca / - homo del) in the fifth segment of the gene showed the highest incidence among patients' samples. Therefore, this change may be considered as a diagnostic indicator of beta-thalassemia (Mishra et al., 2012).

We also found that this change [C. (Bircan et al., 1993; Bolaman et al., 2001) CT //--homo del] occurred in exon area, this in agreement with Kollia et al (Kollia et al., 1989).

In this study, splenectomy in the β-Thalassemia patients led to a lowering blood transfusions in 20% who had spleen removed, and this was consistent with agreed with this study (Al-Salem and Nasserulla, 2002).

The changes in the LCR-HS2β region had the most apparent effect and severity of clinical symptoms (G<T het8966) in β-LCR3 (LCR - HS2) (β-). These results are in agreement with Seoyeon et al (Seoyeon Kim et al., 2012).

When studying the links between the changes in the beta-globin and the changes in the of LCR control region gene in patients with beta by statistical analysis it was found that this is the most sensitive region (LCR - HS2 (β- in L-LCR) with a confidence level of 99%.

It was also found that the in the (LCR - HS2) β- had an effect on the hemoglobin F ratio, as it increases number of patients, with 99% confidence. This finding was consistent with Asadov et al (Asadov et al., 2017).

The MLDA results showed that the deletion of the promoter of exon 3A is the most important section in the beta globin gene linked to high incidence of the disease in β-Thalassemia patients. This was in agreement with Colosimo et al (Colosimo et al., 2011).

In conclusion, this study was unique to show several new mutations that would help in diagnosis and treatment of such common inherited hematologic disease SCA affects on many organs, causing many complications. The results should be taken further to set up better management strategies to improve outcomes.

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