Original article

Biochemical and Molecular analysis of the beta-globin gene on Saudi sickle cell anemia

Faris Q. Alenzi [a,*], Dalal S. AlShaya [b]

[a] College of Appl. Med. Sci., Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia
[b] Dept. of Biology, College of Science, PNU, Riyadh, Saudi Arabia

ABSTRACT

Sickle cell anemia (SCA) is one of the most common hematologic diseases affecting humans. Detection of a single base pair mutation at 6th codon of β-globin gene is important for the diagnosis of SCA. The aim was to study the nucleotide sequences and the molecular survey of β-globin gene in Saudi patients.

Blood samples from 77 unrelated SC patients were obtained from the KKUH, between 2015 and 2017. In this study, DNA was extracted then PCR was performed. Twelve overlapping fragments covering β-globin gene, have been generated by PCR.

A total of 47 alterations have been recognized in β-globin gene. These alterations composed of: deletions, insertion or substitutions as follows:- one mutation identified on the 1st segment; three alterations on 2nd fragment; two alterations on 3rd segment; seven alterations on 4th segment; three substitution on 5th fragment; two changes on 6th fragment; five alterations on 7th fragment; seven substitution changes on 8th fragment; two heterozygous substitution changes on 9th fragment; three changes on 10th fragment and eight substitution changes on 11th fragment, and four changes on 12th fragment.

SCA had profound negative effects on many organs, causing many complications. The results should be taken further to set up management strategies to improve outcomes.

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1. Introduction

Sickle cell disease (SCD) is a major health problem in many countries around the world. According to the World Health Organization (Weatherall and Clegg, 2001; Williams and Weatherall, 2012; WHO), every year nearly 300,000 to 500,000 infants are born with severe haemoglobin disorders, and more than 200,000 people born with sickle cell anaemia. These diseases were found to be less prevalent in southern India, Saudi Arabia, Mediterranean countries and Middle Eastern countries.

Hereditary blood diseases like SCD are caused by molecular changes in specific blood proteins called globins. Globins are the main component of haemoglobin, found in red blood cells. Haemoglobin is made up of four chains of globins, each of which carries the heme group, which is made up of Fe + Fe, which is associated

with oxygen (Schechter, 2008). SCD is one of the most serious of the red blood cell disorders, and is caused by a abnormal haemoglobin known as HbS (Ashley-Koch et al., 2000). While normal red blood cells can survive for up to 120 days, sickle cells die in just 20 days. SCD results from a basal substitution of adenine A to thymine T in the sixth codon of beta-globin. The mutation of a single base in the DNA leads to the replacement of glutamic acid with valine in the polypeptide of the beta-globin chain in the haemoglobin S (HbS).

SCD is characterized by chronic haemolysis, recurrent vasoconstriction, rapid infection, failure of various organs in the body, a periodic pain, inflammation, stroke, and acute chest pain, abnormal haemoglobin in red blood cells, causing them to lose their flexibility and turn into the form of solid sickles (Lionne et al., 2012). Chronic haemolysis can lead to varying degrees of anaemia, jaundice, biliary tuberculosis, delayed growth and sexual maturity. Patients are also sensitive for the highest rates of pulmonary arterial haemorrhage, hypertension, rheumatism and leg ulcers (Bender and Hobbs, 2003; Smith-Whitley and Sickle, 2007; Ballas et al., 2012; Abbas et al., 2013).

In Saudi Arabia, the SCD was first identified in the eastern region in 1960 by Lehman (Lehaman et al., 1963; Weatherall, 2010), which led to the initiation of multiple studies at the regional and national level to determine the clinical characteristics and gene replication of SCD in different regions of Saudi Arabia. SCD

is more common hereditary disorder in this part of the world, ranging from 0-4-8% while SCD carriers ranged from 2% to 27%. These estimates do not include neonatal screening, which may increase the real frequency of the disease (Jastaniah, 2011). Statistical analysis showed that there are about 3 million people had these genetic blood diseases in the Saudi Arabia, of which 30% in the Eastern Region (Al-Naseri, 2009). El-Hazmi and others reported two different types of sickle cell anaemia, the average type of acute disease in most patients in the Eastern Region, while the severe type was observed in most patients in the southwestern region (El-Hazmi, 1979; El-Hazmi, 1983; El-Hazmi, 1985; El-Hazmi et al., 1990; El-Hazmi et al., 1991; El-Hazmi, 1992; El-Hazmi and Warsy, 1999; Al-Qurashi et al., 2008).

The aim was to study the nucleotide sequences and the molecular survey of the β-globin gene in Saudi patients with sickle cell anemia, and to identify the genetic variants associated with clinical manifestations.

2. Materials and methods

2.1. Patients

The study was conducted on 77 of sickle cell anemia patients selected randomly from the attending the blood diseases clinic at King Khalid University Hospital (KKUH, Riyadh, KSA) from different regions of Saudi Arabia between Jan 2015 - June 2017. 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 for all cases.

2.2. Sample

Ten ml of venous blood was withdrawn from each patient and distributed to two tubes (each containing 5 ml) of ethylenediamine tetra acetic acid (EDTA). The analyzers used in this study were Advia 2120 from Siemens Company for measuring hematology markers and Architect from Abbott Company for measuring biochemical markers.

2.3. Extraction of DNA

DNA was extracted using a Qiagen gel purification kit, according to the manufacturer's instructions.

2.4. Primers & PCR

Primers were designed, requested and obtained through the Oligo ordering online. PCR primers were used (see Table 1). 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 OmniGene; 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 percent 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).

2.5. Statistical analysis

The data obtained was subjected to a statistical analysis using Window Excel and SPSS v17 statistical tools. ANOVAs tests for multiple comparisons and significant analysis ($p < 0.05$) were carried out.

Table 1
The primers that were designed and used in this study to multiply the beta-globin gene.

Gene/Fragment	Primer seq 5′ – 3′	Product size (b.p)
HBB-1F	Gtatgcctgggcttttgatg	482
HBB-1R	Ccctgtttcacatccctgat	
HBB-2F	Gcattttctttgacccagga	488
HBB-2R	Acctgtctcaaccctcatca	
HBB-3F	Ccaaacactttctgcgtgtg	494
HBB-3R	Gcagagttctgcttcctgct	
HBB-4F	Tgagacccttacgctgacctc	458
HBB-4R	Caatggggtaatcagtggtg	
HBB-5F	Ttttgttcccccagacactc	471
HBB-5R	Tcttcctgcgtctccagaat	
HBB-6F	Tttttctttttcttaccagaaggtttt	470
HBB-6R	Tgctcctgggagtagattgg	
HBB-7F	Actcctaagccagtgccaga	490
HBB-7R	CAGATCCCCAAAGGACTCAA	
HBB-8F	Ggcactgactctctctgccta	496
HBB-8R	Aaaaattgcggagaagaaaaa	
HBB-9F	Tgttttctttttgtttaattcttgcttt	486
HBB-9R	Ttgctattgccttaacccaga	
HBB-10F	Ttcagggcaataatgatacaatg	500
HBB-10R	GATGCTCAAGGCCCTTCATA	
HBB-11F	TTCCTTTGTTCCCTAAGTCCAA	489
HBB-11R	Ggaacacttcaggggaaagg	
HBB-12F	Tgcatctctcagccttgact	600
HBB-12R	Cttgagactcatattttatttccaga	

3. Results

The study was conducted on 77 patients with sickle cell anemia attending blood diseases clinic at King Khalid University Hospital (KKUH) from different regions of Saudi Arabia. A comprehensive database was created including the patient's name, patient history, and biochemical and hematological tests (Table 2). In addition, a number of modern and advanced molecular techniques were used to identify the genetic basis of the disease through the molecular survey of the nucleotide sequences of beta-globin and the entire gamma control areas within chromosome 11. Fig. 1 shows the nature of the patients with sickle cell anemia. 82% of the patients were homozygous, 14% were gametes, and 4% had thalassemia.

3.1. Clinical and blood data in the research sample:

Table 2 presents a summary of hematological and biochemical measurements from patients with sickle cell anemia that have been followed for more than 30 months.

3.2. WBC count

As shown in the Table 3, there was a significant difference between those individuals with HBBF8_3 mutation and who don't. In other words, the presence of this mutation may cause an increase in the WBC count compared-globin

3.3. Hemoglobin level

As shown in Table 4, there was a significant difference between those with mutations in HBBF4-3, HBBF5-1, HBBF8-2, HBBF12-1, HBBF12-3, compared to the control group.

3.4. Platelets

As shown in Table 5, there was a not statistical difference between those with mutations and the control group.

Table 2
Hematological and biochemical measurements from SCA patients.

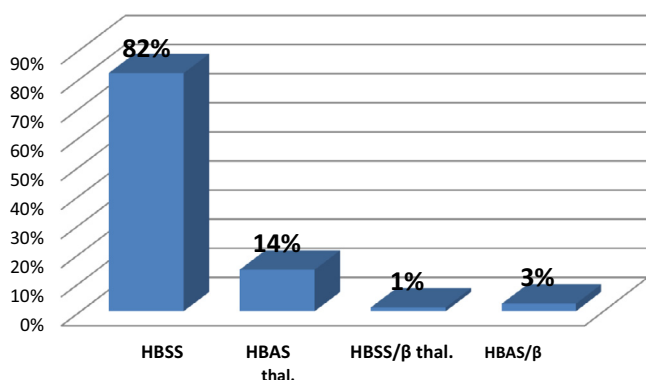|		WBC	Hgb	Plt	LDH	Bilirubin
Mean		11.33	91.67	368.24	446.24	48.18
Median		10.55	95	346	432	37
Std. Deviation		4.89	23.02	165.67	206.40	40.06
Minimum		0.3	8.5	44	136	6
Maximum		31	136	777	1000	243
Percentiles	25	8.53	81.5	260.75	282.33	29.5
	50	10.55	95	346	432	37
	75	14.23	106.3	467.75	584.75	52.67

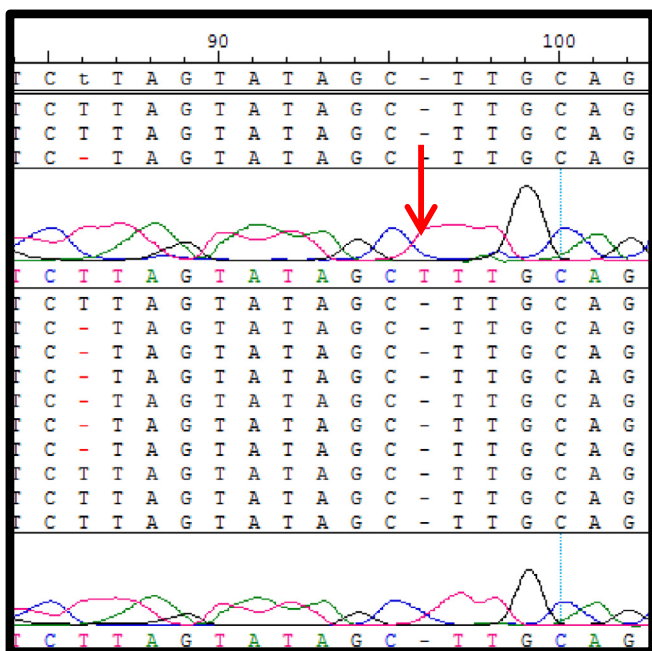Fig. 1. Patients characteristics on this study.

Fig. 2. Adding the T-base indicates the arrow to the location specified. * C.-2454- / t Ins.

3.5. Level of lactate dehydrogenase (LDH)

As shown in Table 6, there was a significant difference between those with mutation in HBBF9-1, HBBF12-1, compared to the control group.

3.6. Bilirubin level

As shown in Table 7, there was a significant difference between those with a mutation in HBBF9-1 compared to normal individuals (i.e, this mutation can increase bilirubin level).

Table 3
Relationship between WBC count and mutation.

Part/ Variable		WBC		
		Mean	Median	Standard Deviation
HBBF2_2	Normal	11.16	10.50	5.08
	Mutated	12.63	11.50	2.94
HBBF4_3	Normal	11.26	10.55	5.09
	Mutated	11.84	10.20	3.19
HBBF5_1	Normal	11.27	10.50	4.84
	Mutated	12.50	14.60	6.99
HBBF7_4	Normal	11.42	10.60	4.94
	Mutated	9.95	9.35	4.48
HBBF8_2	Normal	11.13	10.50	5.03
	Mutated	13.17	12.75	3.06
HBBF8_3	Normal	10.72	10.45	4.31
	Mutated	14.85	14.00	6.67
HBBF9_1	Normal	11.11	10.50	5.05
	Mutated	13.00	13.60	3.16
HBBF12_1	Normal	11.32	10.55	4.92
	Mutated	11.40	10.20	5.04
HBBF12_2	Normal	11.39	10.74	4.99
	Mutated	10.81	9.90	4.38
HBBF12_3	Normal	11.33	10.60	5.03
	Mutated	11.33	10.35	4.16

Using Mann-Whitney test to determine whether there is a relationship between the parameters including: WBC, HB, PLT, LDH, Bilirubin and those individuals with mutations, Table 8 showed significant effect of some mutations on those individuals examined Hb, Platelets, and Bilirubin.

In this study, the beta-globin gene was divided into 12 overlapping segments covering all regions of the gene from exons and introns (Table 2, Figs. 3–13). The molecular assessment of the entire gene fraction was performed and compared to the gene reference using DNA star-Lasergene® software. The primary disease mutation was confirmed in all patients at the beginning of the seventh piece Hbb / Fragment 7: (C.20 GAG > GTG A > T EtoVRs # 77121243). These changes were widely varied between substitution in different nitrogen bases, either by deletion, or Insertion. The number of changes identified in the beta-globin area was 47. These changes were matched by the mutations documented in the global database at: (http://www.ensembl.org) to identify new changes that do not have a reference number (RS).

Table 4
Relationship between Hb level count and mutation.

Part/ Variable		HB_or_Hgb		
		Mean	Median	Standard Deviation
HBBF2_2	Normal	92.70	96.00	23.85
	Mutated	83.71	87.00	13.86
HBBF4_3	Normal	92.74	96.00	24.08
	Mutated	83.43	83.00	9.32
HBBF5_1	Normal	92.38	96.00	23.35
	Mutated	78.00	74.00	7.81
HBBF7_4	Normal	90.63	94.00	23.15
	Mutated	106.50	104.00	16.76
HBBF8_2	Normal	92.89	96.00	23.68
	Mutated	80.50	80.50	11.74
HBBF8_3	Normal	94.03	96.00	20.24
	Mutated	78.03	83.00	33.43
HBBF9_1	Normal	92.01	95.50	23.93
	Mutated	89.00	89.00	15.21
HBBF12_1	Normal	92.87	96.00	23.56
	Mutated	78.20	74.00	8.29
HBBF12_2	Normal	91.76	95.50	23.83
	Mutated	91.00	87.00	16.78
HBBF12_3	Normal	92.77	96.00	24.19
	Mutated	84.38	86.00	11.24

Table 5
Relationship between Platelets count and mutation.

Part/Variable		Platelets		
		Mean	Median	Standard Deviation
HBBF2_2	Normal	368.45	343.50	159.60
	Mutated	366.57	431.00	222.42
HBBF4_3	Normal	372.89	354.00	171.03
	Mutated	332.36	341.00	119.97
HBBF5_1	Normal	368.99	354.00	157.90
	Mutated	353.67	307.00	335.44
HBBF7_4	Normal	373.38	346.00	164.59
	Mutated	295.00	293.50	188.76
HBBF8_2	Normal	370.48	346.00	158.72
	Mutated	347.67	322.00	238.19
HBBF8_3	Normal	363.36	343.50	171.88
	Mutated	396.44	366.00	128.30
HBBF9_1	Normal	372.78	363.50	170.17
	Mutated	333.21	341.00	130.56
HBBF12_1	Normal	372.42	354.00	161.18
	Mutated	321.40	307.00	226.91
HBBF12_2	Normal	358.44	343.50	151.02
	Mutated	443.86	488.00	255.90
HBBF12_3	Normal	366.52	346.00	156.89
	Mutated	379.63	359.50	228.53

Table 6
Relationship between LDH level and mutation.

Part/ Variable		LDH		
		Mean	Median	Standard Deviation
HBBF2_2	Normal	540.43	431.50	669.86
	Mutated	523.67	609.00	231.13
HBBF4_3	Normal	540.16	426.00	696.33
	Mutated	533.67	517.00	174.13
HBBF5_1	Normal	537.71	431.00	663.37
	Mutated	571.00	571.00	53.74
HBBF7_4	Normal	546.08	431.50	670.17
	Mutated	448.33	441.00	180.11
HBBF8_2	Normal	531.34	431.00	676.11
	Mutated	616.50	654.50	264.74
HBBF8_3	Normal	556.95	438.00	687.20
	Mutated	404.82	432.00	89.24
HBBF9_1	Normal	524.84	362.00	705.58
	Mutated	613.43	632.00	150.89
HBBF12_1	Normal	528.78	428.50	669.32
	Mutated	679.00	609.00	190.88
HBBF12_2	Normal	550.87	432.00	677.90
	Mutated	426.00	416.50	170.99
HBBF12_3	Normal	535.62	431.00	676.65
	Mutated	574.75	571.00	260.31

Table 7
Relationship between Bilirubin level and mutation.

Part/ Variable		Bilirubin		
		Mean	Median	Standard Deviation
HBBF2_2	Normal	44.36	37.00	28.82
	Mutated	80.00	36.00	90.32
HBBF4_3	Normal	49.51	37.00	42.36
	Mutated	38.86	42.00	15.67
HBBF5_1	Normal	47.06	37.00	39.11
	Mutated	78.50	78.50	72.83
HBBF7_4	Normal	47.90	36.50	41.33
	Mutated	51.75	49.50	19.31
HBBF8_2	Normal	43.82	37.00	28.74
	Mutated	92.60	42.00	95.07
HBBF8_3	Normal	50.08	39.00	42.47
	Mutated	36.75	33.50	18.09
HBBF9_1	Normal	44.39	35.00	38.43
	Mutated	74.71	59.00	44.27
HBBF12_1	Normal	48.04	37.00	39.96
	Mutated	49.60	33.00	45.93
HBBF12_2	Normal	48.38	38.00	40.11
	Mutated	46.50	32.00	43.41
HBBF12_3	Normal	48.59	37.00	40.49
	Mutated	45.29	33.00	39.84

One change was identified in the first segment of the beta-globin gene (Fig. 2), which represents the introduction or addition of the thymine (T) base to the original gene sequence at site 2454 and was found in one patient at (1.3%: c.-2454- / T homo ins). A new change not previously registered in the global reference database.

The second segment of the gene was found to have three changes: heterozygous replacement of adenine base with the cytosine base (het. * C- 1917c > t) in about 10% of our samples, homol-ogous replacement at the same site by (1.3% homo. 1917c > t), and heterozygous replacement of (het. * C.- 1934c > a), replacing the

Table 8
Relationship between Lab parameters and SCD mutation.

Part/ Variable	Significance Level (P- Value) one tail				
	WBC	HB	PLT	LDH	Bilirubin
HBBF2_2	0.125	0.051	0.4955	0.2855	0.4845
HBBF4_3	0.333	0.034 *	0.374	0.1385	0.4615
HBBF5_1	0.312	0.044 *	0.3465	0.146	0.3585
HBBF7_4	0.254	0.0815	0.2725	0.5	0.173
HBBF8_2	0.084	0.022 *	0.366	0.098	0.242
HBBF8_3	0.032 *	0.061	0.288	0.338	0.177
HBBF9_1	0.081	0.224	0.362	0.009 *	0.003 *
HBBF12_1	0.469	0.015 *	0.223	0.032 *	0.327
HBBF12_2	0.350	0.278	0.195	0.467	0.271
HBBF12_3	0.474	0.041 *	0.483	0.148	0.268

adenine base with the cytosine base. New changes were found namely (-1119 G > A homo) repeated in eight patients, followed by heterozygous change in the same location (* c. -1119 G > A het) repeated in three patients, followed by homozygous change (* C. -1039C > G) repeated in two patients. In the sixth segment of the gene, two changes were identified in the same location; former one is recorded at high frequency (in 96%: c-390 C > T homo),
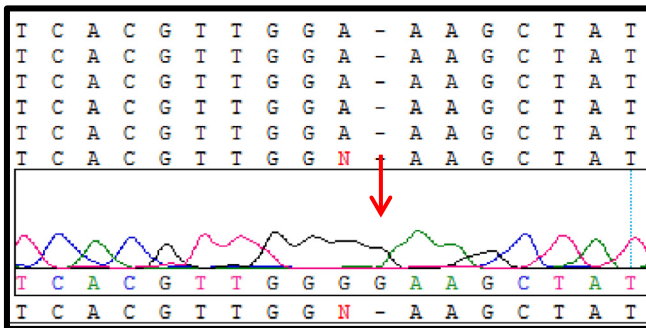
Fig. 3. A new change identified in the fourth piece of the beta-glubin gene, the addition of base G at the indicated site * C. -1432–14333 - / G Ins.
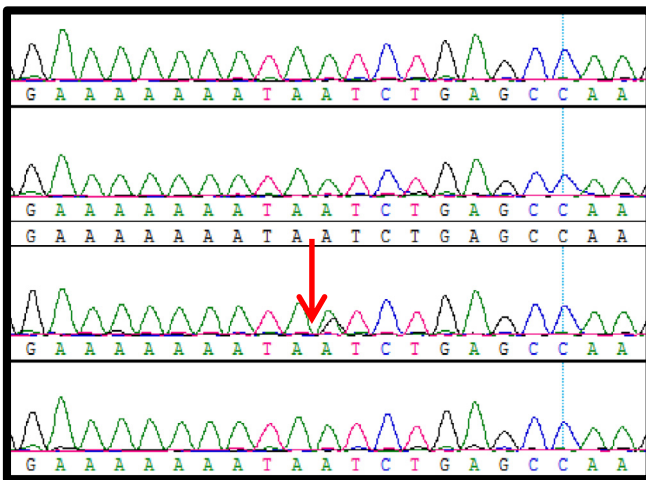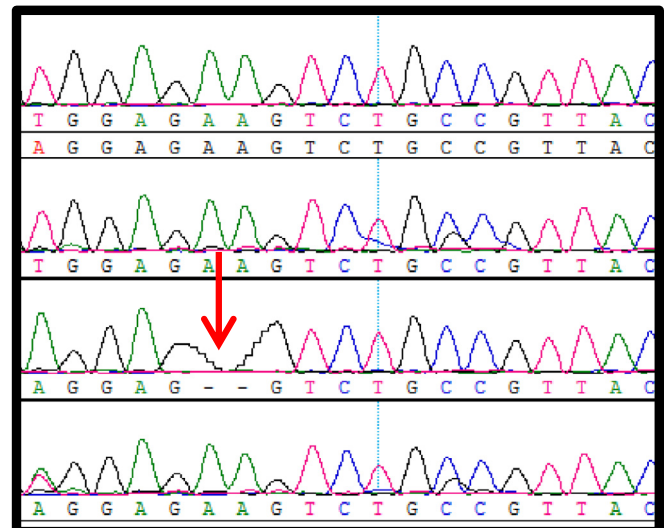

Fig. 4. A change identified in the fourth piece of the beta-gluten gene, a homozygous homozygous replacement of base G at base A at the indicated location * C. -1119 G > A Rs. 1,003,586.
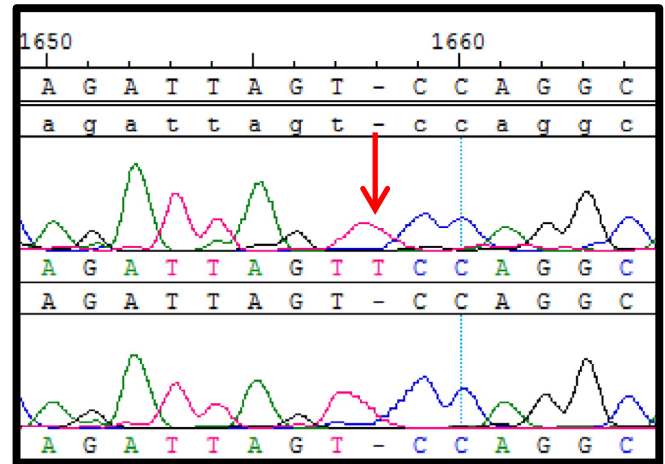
Fig. 5. A change identified in the fourth piece of the beta-gluten gene, a heterozygous replacement of base G of base A at the indicated location * C. -1119 G > A Rs. 1,003,586.
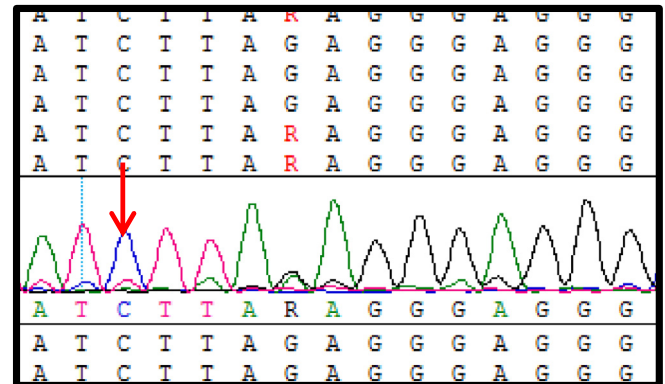
Fig. 6. Changes identified in the sixth segment of the beta-gluten gene, a substitution between two bases at the specified location *c-240 G > A.

Fig. 7. A new change was identified in the seventh piece of the beta-globin gene, a deletion in two bases at the specified location C.25 to27 AA / - homo del.
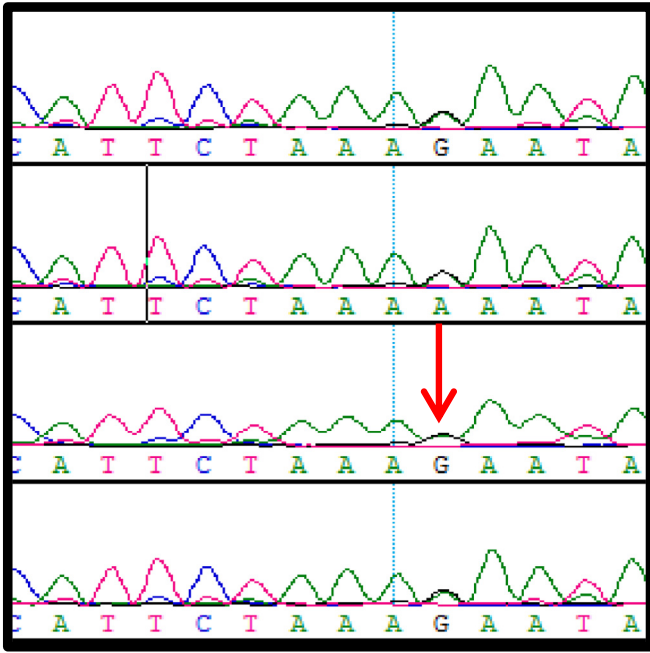
Fig. 8. A new change identified in the ninth piece of the beta-gluten gene, a heterozygous replacement of the gamma-G base of the adenine A base at the specified location C.316-225G > A.
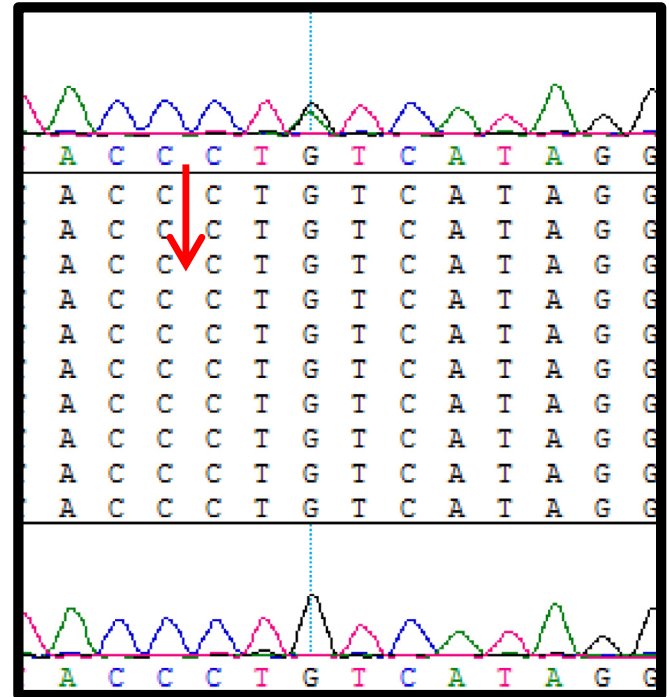

Fig. 10. A new change identified in the tenth piece of the beta-gluten gene, which is a replacement of the G-cell base G with the thymine T base at the specified location C.212 G > T.
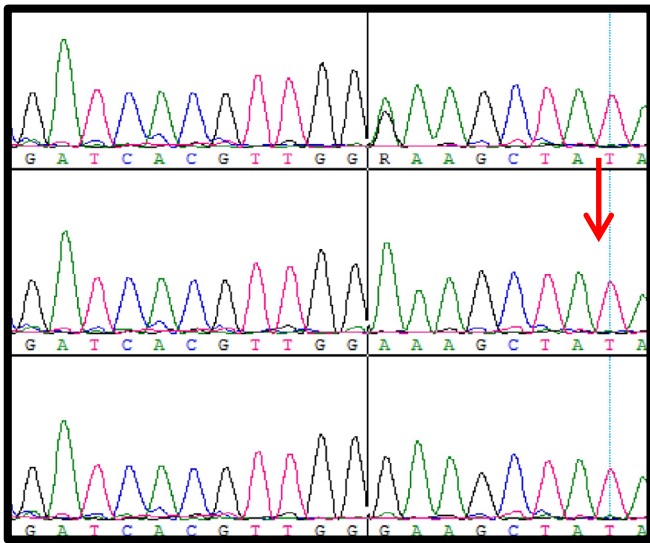

Fig. 9. A new change identified in the ninth piece of the beta-gluten gene, a heterozygous replacement of the gamma-G base of the adenine A base at the specified location C.315 + 282G > A.
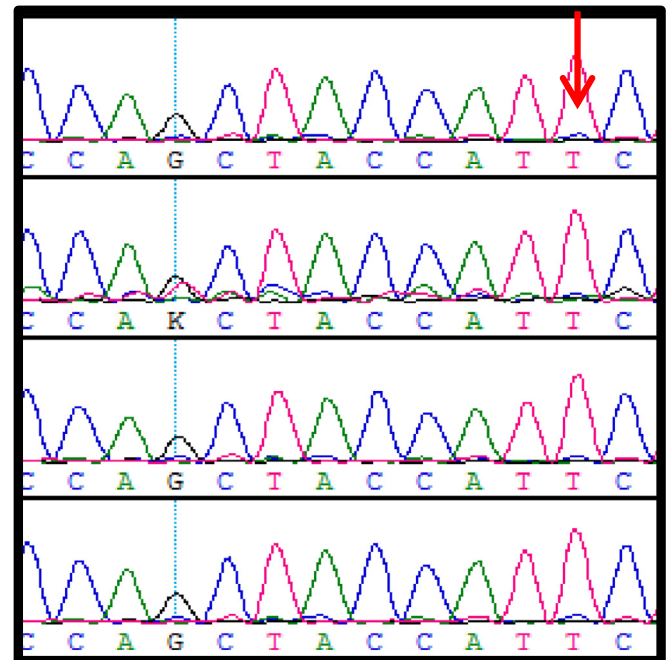

Fig. 11. A change identified in the tenth piece of the beta-gluten gene, which replaces the adenine A base with the Gouanine G base at the specified location * C. + 828 G > A.

the later is (4%: * c-390 C > T het). The seventh segment of beta-globin was an important discovery in this study, the mutation was found in all subjects of our study 100%. The changes are new and have not been recorded in the reference database, which are deletion of two bases (c.25 to 27 AA / - homo.del. −2544 G > A), while the changes were made (c.118 CAG > TAG C > T Q40) end, (c. 93 + 1 G > C) and found in three patients. While the ninth segment has two new changes that were not recorded previously namely: heterozygous substitutions in two different locations (c.316-225G > A het) and (het.c.315 + 282G > A). In the tenth segment, two replicates were found in all individuals of our samples (100%, c.316–185 C > T het). This change was a silent mutation: (het. C.396 CAG > CAA G > A Q132Q).

4. Discussion

In the past three decades, many studies have focused on the clinical and biochemical features of SCD in Saudi patients, some of which have targeted specific mutations in beta-globin in Saudi patients (El-Hazmi, 1979; El-Hazmi, 1983; El-Hazmi, 1985; El-Hazmi et al., 1990; El-Hazmi et al., 1991; El-Hazmi, 1992;
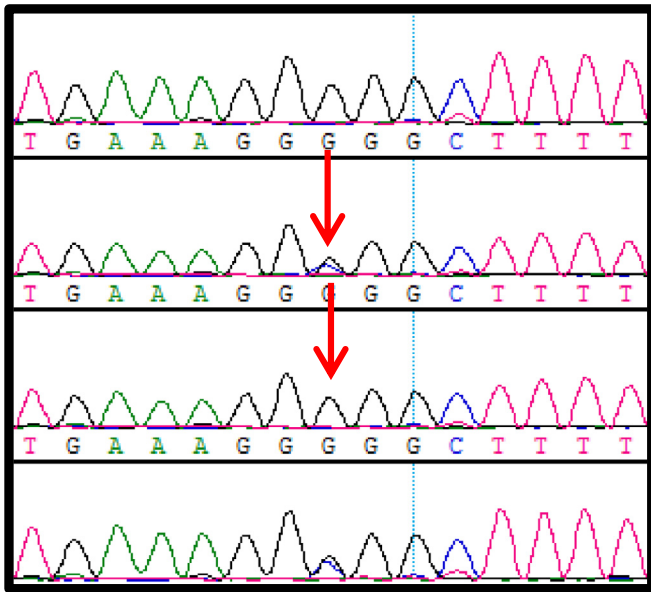
Fig. 12. A change was found in the twelve part of the beta-glubin gene, a replacement of the G base with the C base at the specified location *C. + 786C > G.

El-Hazmi and Warsy, 1999), while Al-Shehri's group examined the whole beta-globin gene (Al-Shehri, 2011) to identify the specific mutations in Saudi patients. Our current study was designed to investigate the complete β-globin gene, which is about 3.8 Kb including UTR untranslated regions 5 and 3, and all oxons and introns, in order to identify all mutations and genetic changes in SCD.

El-Tayeb et al. (2008) reported interesting results in his study on the prevalence of sickle cell anaemia and thalassemia in Al-Qassim region, where they showed that the prevalence of thalassemia beta and sickle cell anaemia to be 0.165% and 0.252% respectively. Ballas et al. (2012) also showed that the clinical manifestations of sickle cell disease vary widely among patients, where some patients with sickle cell anaemia have mild disease, while others suffer from a severe form and relatively high mortality at early age.

Our current results were obtained from 77 SCD patients at the KKUH from various regions of Saudi Arabia, exhibiting a wide spectrum of the disease from mild to severe stages. This was evidenced by the follow-up of several indicators in patients' records throughout the study period. The differences in SCD patients were related to the need for medical emergency assistance, as well as the need to stay at the hospital for several days to receive the necessary treatment.

During the follow-up of 48 SCD patients, we monitored more than 69 pain crisis in 21 individuals. The vast majority of follow-up SCD patients were treated at the emergency department, while a few were admitted to the hospital. It was clear that the clinical symptoms of our patients vary greatly, as reported by Quimby et al. (2014).

The beta-globin gene is located on chromosome 11 and consists of three exons separated by two interconnected sequences of introns known as "IVS". Mutations at introns (IVS-1, IVS-2) may have a significant effect on the gene expression of the beta-globin gene (Warsy et al., 2013).

The genetic survey of the beta-globin gene in our sample showed a wide range of changes (48 changes including: eight new changes not included in the global database). These changes varied widely between different nitrogen bases (c. + 828 G > A

het) , (c. + 786 C < G homo) and (c. + 786 C < G het). It was found that the last three changes Were new and did not have reference figures. It was also found that some of these observed mutations were statistically and strongly correlated with one or more of the clinical symptoms.

A close relationship between the clinical homogeneity of SCD and haemoglobin levels was found (40.16%. -1119 G > A homo./het. Rs # 1003586). LDH is a clinically significant indicator of haemolysis and was associated with a decreased haemoglobin levels in our patients, (Banihashemi and Azizi, 2012). Moreover, the bellowed changes were also associated with an increase in the WBCs count in our study: (rs # 10768683c. 315 + 16 G > C homo).

Préhu's group suggested a strong relationship between low oxygen levels, SCD and this change (1119 G > A homo./het. Rs # 1003586), and this has been supported by a previous study (Préhu et al., 2002), which also linked the same change with an increase in the number of white blood cells (WBCs).

The change (rs # 10,742,583 * c-390 C > T homo./het), was demonstrated in the sixth segment of the gene in all patients, but did not appear in the control group, while the change (rs # 34188626c.396 CAG > CAA G > A Q132Q het), in the tenth segment, appeared to affect the severity of the disease, and is a silent mutation.

In the eighth segment of the gene, the change (c.315 + 1 G > A het), was related to the levels of HbF, which has a role in improving the clinical severity of SCD patients. There were also new changes in the eighth region among our patients but not in the control group by 3.9%: (c.315 + 26 T > G het), (c.315 + 1 G > A het) and (c.- 1039C > G homo./het.rs# 16911905). In the twelfth segment of the gene, there was a new change that has not been observed in the Global Human Genome Database (100%) in both groups. New unregistered changes in the database were found by 1% in the study: (*c.-2454-/t homo ins), (*c. −1432–14333 -/G homo ins), (*c.-986 A > G het), (*c753 T < C homo), (c.25 to27 AA/-- homo del), (*c. −2544 G > A het), (*c. −2544 G > A het), (c.315 + 282G > A het), and (c.212 G > T het).

Another change was demonstrated in the tenth segment of the gene in all patients as well as the control group (c.316–185 C > T het). This change has been confirmed by Trecarinet's study, where they investigated the genetic survey of thalassaemic patients from Mediterranean and Turkish individuals (Trecartin et al., 1981).

Ahmed's group showed elevation in WBC count in 50% of SCD patients and were with lowered Hb concentration. They concluded that a special strategies to lower WBC in order to prevent disease complications (Ahmed et al., 2017). Habara et al. reviewed the genetic basis and disease severity of SCD and suggested that huge efforts to be made for the treatment and counseling (Habara and Steinberg, 2016). Damanhouri et al showed that SCD patients have many clinical features such as: hypercoagulapathy, hemolysis, inflammation, oxidation stress, vasculopathy and reduced vasodilatory response. Their blood counts and urine are similar to ours (Damanhouri et al., 2015). Steinberg et al found 4 SNPs namely (rs3834466; rs28440105; rs10128556; rs968857) using RFLP technology (Shaikho et al., 2017). Alsultan's group assessed 104 SCD patients and found that 96% have pain, 47% have chest infection, 18% have osteonecrosis, 66% with gallstone, 33% with G6PD deficiency, and 7% with stroke (Alsultan et al., 2014). Kalai et al found that rs1984112 is associated with high reticulocytosis count in SCD patients, allowed earlier and reliable biomarkers for vascular occlusions (Kalai et al., 2017).

In conclusion, SCD had profound negative effects on many organs, causing many complications. The above results should be taken further to set up management strategies to improve outcomes.

Acknowledgments

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