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

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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; Chia-Cheng and Shee-Uan Chen; Shin-Yu Lin; Mei-Ya Fang; Li-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10 ml of venous blood was withdrawn from each patient and distributed to two tubes (each containing 5 ml) of ethylenediamine tetra acetic acid (EDTA) and kept at 4°C for later use.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 (Hybraid 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% 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 mut-
multiple 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 \(\beta\)-Thalassemia patients

In order to fully understand and diagnose \(\beta\)-Thalassemia, there are many techniques, however, we made a full screening for nucleotide sequence plus 6 LCR regions by using PCR to indentify all exist- ing mutations, later, we used a revusionalized techniques name as Multiplex ligation dependent probe amplification (MLPA). We identified two changes; those in \(\beta\)-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 \(\beta\)-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 \(\beta\)-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 \(\beta\)-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 \(\text{c-390C}<\text{T homo}\) and WBC count and Ferritin, as shown in Table 9.
   A significant relationship was found in segment no-6 between \(\text{C-1039C}<\text{G homo & C-390C}<\text{T homo}\) and LDH and Bilirubin, as shown in Table 10.
   A significant relationship was found in segment no-6 between \(\text{C-1039C}<\text{G homo}\) and splenoectomy, as shown in Table 11.

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

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

LCR region of \(\beta\)-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:

Table 3

| Primer | Primer seq 5’-3’ | Product size (b.p) |
|--------|------------------|--------------------|
| LCR-1F | CTGCAACGTATCTGGTCAC | 445 |
| LCR-1R | CTAGCGGGTATTTTATTTGTT | 460 |
| LCR-3F | ATGGCCGACACGGAGGCCATAGGC | 595 |
| LCR-3R | CCAACACACACACACACGACG | 442 |
| LCR-4R | CAGGGGACCCATCTCATAGGC | 544 |
| LCR-5F | GCCCTCTCCCATACACTAC | 520 |
| LCR-5R | ATGGCCGACACGGAGGCCATAGGC | 539 |
| LCR-7F | TCAGGGGAACTTTGTTACCATATAA | |

Table 4

New changes obtained from \(\beta\)-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% |
| HBB/F3 | c.1271 G-A | Het | No-Ref Number | 16.4% |
| HBB/F4 | c.870C<T | Het | No-Ref Number | 3.5% |
| HBB/F5 | c.559 G>A | Het | No-Ref Number | 3.5% |
| HBB/F6 | c.536 G>A | Het | No-Ref Number | 3.5% |
| 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% |
| HBB/F12 | c.316-225G<A | Het | No-Ref Number | 100.0% |
| c.315+342 G<A | Het | No-Ref Number | 3.5% |
| c.316-225G<A | Het | No-Ref Number | 3.5% |
| c.316-225G<A | Het | No-Ref Number | 3.5% |
| c.315+342 G<A | Het | No-Ref Number | 3.5% |
| c.315+342 G<A | Het | No-Ref Number | 3.5% |
| c.315+342 G<A | Het | No-Ref Number | 3.5% |
| c.316-225G<A | Het | No-Ref Number | 3.5% |
| c.316-225G<A | Het | No-Ref Number | 3.5% |
| c.316-225G<A | Het | No-Ref Number | 3.5% |
| c.315+342 G<A | Het | No-Ref Number | 3.5% |
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 | Ins      | Rs# 59124155 | 6.4%  |
|                 | 1090–1130 | Del       | Rs# 6168339  | 3.5%  |
| HBB/F4          | C-1119 G>A | Homo      | Rs# 1033596  | 13.5% |
|                 | C-1039C>G | Homo      | Rs# 16911905 | 33.5% |
| HBB/F5          | C-601 T>C | Het       | Rs# 33575129 | 30.0% |
|                 | C-601 T>C | Het       | Rs# 1103634  | 30.5% |
|                 | C-753 T>C | Het       | Rs# 5489254  | 3.5%  |
|                 | C-1980–1981 | Ins     | Rs# 67721287 | 3.5%  |
|                 | C-518C>T | Het       | Rs# 10742584 | 3.5%  |
| HBB/F6          | C-1119 G>A | Homo      | Rs# 1003586  | 13.5% |
|                 | C-1039C>G | Homo      | Rs# 16911905 | 33.5% |
|                 | C-601 T>C | Het       | Rs# 33575129 | 30.0% |
|                 | C-753 T>C | Het       | Rs# 5489254  | 3.5%  |
|                 | C-1980–1981 | Ins     | Rs# 67721287 | 3.5%  |
|                 | C-518C>T | Het       | Rs# 10742584 | 3.5%  |
| HBB/F7          | C. (17_18) | CT/-  | Del          | Rs# 63750729 | 3.5%  |
|                 | C.92+5 G>C | Homo | Rs# CS820004 | 6.4% |
|                 | C.92+6 T>C | Homo | Rs# 35724775 | 6.4% |
|                 | C.118 CAG>TAG C>T 40end | Homo | Rs# CM810001 | 16.4% |
| HBB/F8          | C.315+81 C>T | Homo | CS870042 | 16.4% |
|                 | C.315+1 G>A | Homo | Rs# TMP_ESP_11_5247781 | 16.4% |
|                 | C.315+26 T>G | Homo | Rs# 121909815 | 90.0% |
|                 | C.316–185C>T | Homo | Rs# 10768683 | 85.0% |
|                 | C.316–185C>T | Homo | Rs# 7496748 | 25.0% |
|                 | C.396 CAG<TAG C>T Q132Q | Homo | Rs# 7496748 | 25.0% |
| HBB/F11         | C.233 G>C | Homo | Rs# 12788013 | 33.5% |
|                 | C.472 A>T | Homo | Rs# 10837631 | 20.0% |
|                 | C.472 A>T | Homo | Rs# 10837631 | 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/F5          | C-1960–1961 | ca/– | Homo | Rs# 10742584 | 100.0% |
| HBB/F6          | C-519C<T | Homo | Rs# 10742583 | 80.5% |
|                 | C-390C<T | Homo | Rs# 10742583 | 80.5% |
| HBB/F7          | C.9 C>T 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# 7496748 | 60.0% |
| HBB/F10         | C.316–185C>T | Homo | Rs# 1609812 | 26.4% |
| HBB/F11         | C.316 A>C | Homo | Rs# 7110263 | 70.0% |
|                 | C.316 A>C | Homo | Rs# 7110263 | 70.0% |
| HBB/F12         | C.625 T>G | Het    | Rs# 78928216 | 36.4% |
|                 | C.625 T>G | Het    | Rs# 78928216 | 23.5% |

Table 8
Relationship between this change (‘C-1271 G>A) of β-Thalassemia patients and their Lab investigations’ results.

| Mutation | P value |
|----------|---------|
| WBCs     |         |
| 14.1 ± 4.1 | 0.85 |
| 91.7 ± 4.7 | 0.16 |
| 245 ± 224  | 0.07   |
| 62.1 ± 6.8  | <0.01*|
| 2.71 ± 0.25 | 0.12   |
| 0.72 ± 0.09 | 0.31   |
| 876 ± 620  | 0.64   |

Table 9
Relationship between this change (‘C-1039C>G ho & ‘c-390C<T homo) of β-Thalassemia patients and their Lab investigations’ results.

| Mutation | P value |
|----------|---------|
| WBCs     |         |
| 7.8 ± 1.6 | 0.05*   |
| 96 ± 4    | 0.01    |
| 245 ± 37  | 0.91    |
| 28.6 ± 12.7 | 0.32  |
| 3.97 ± 0.52 | 0.79   |
| 4.3 ± 3.6  | 0.03**  |
| 5136 ± 227 | <0.001**|

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

| Mutation | P value |
|----------|---------|
| WBCs     |         |
| 13.5 ± 1.6 | 0.92    |
| 84.5 ± 2.3  | 0.51    |
| 229 ± 23    | <0.04*  |
| 29.8 ± 4.5  | <0.04*  |
| 3.6 ± 0.3   | 0.58    |
| 3.6 ± 1.4   | 0.59    |
| 1314 ± 539  | 0.61    |
Table 12

| %   | Mutation          | Change | No segment | p     |
|-----|-------------------|--------|------------|-------|
| 4   | Homo              | 64081 A< T | 1           | F-β- LCR7 |
| 98  | Ins               | 63918–63919 <- C | 2 | (start:63386 – 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           |                |

- 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) Table 12

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 (Table 13).

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-Swailim, 1995). Alsuilimi’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 (Alsultan 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 up to 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 C<A homo, C+828 C<A het* , C+786C<G homo, C+786C<G het] (*c-2049T<- del, c-1440–1438 TTT/- del, c-1442-1436 ATTTTG/- 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+38T<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<GAC 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 splenectomy.

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 HBBS and c-519C<T homo, c-390C<T homo) in HBB8. 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.

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There was a positive correlation between the change reported in this study (*-C-2171G>A in the third segment of the beta-globin gene and high Bilirubin level, as this accompanied by 7% lowering blood transfusions in 20% who had spleen removed, and increased number of patients, with 99% confidence. This finding was consistent with Asadov et al (Asadov et al., 2017).

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 - HSR2 (βi- in L-LCR) with a confidence level of 99%.

It was also found that the in the (LCR - HS2) βi- 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 the beta-thalassemia mutations in an Azerbajani population. Turk. J. Haematol. 34 (3), 258–263. https://doi.org/10.2474/tjh.2016.0427.

Bandyopadhyay, A., Bandyopadhyay, S., Chowdhury, M.D., Dasgupta, U.B., 1999. Major β-globin gene mutations in Eastern India and their associated haplotypes. Hum. Hered. 49, 232–235.

Bircan, I., Sisli, S., Guven, A., et al., 1993. Hemoglobinopathies in the district of Antalya. Turkey. Pediatr. Hematol. Oncol. 10, 289–291.

Bolaman, Z., Enli, Y., Koseoglu, M., et al., 2001. Prevalence of Beta Thalassemia trait in Denizli. Turkish J. Haem. 18, 85–88.

Cao, A., Gossens, M., Pirastu, M., 1989. Molecular heterogeneity of beta-thalassemia in the Eastern Province of Saudi Arabia. J. Biomed. Biotechnol. 322–325

Al-Allawi, N.A., Jubrael, J.M., Hughson, M., 2006. Molecular characterization of beta-thalassemia in the Dehok region of Iraq. Hemoglobin 30 (4), 479–486.

Alseade, E.S., Farhat, G.N., Assiri, A.M., Memish, Z., Ahmad, M.Y., Al-Dossary, M.F., Bhashaw, H., 2018. Distribution of hemoglobinopathy disorders in Saudi Arabia based on data from the premartial screening and genetic counseling program, 2011-2015. J Epidemiol. Glob. Health. 2018;7 Suppl 1 (Suppl 1):S41-S47. doi: 10.1016/j.jegh.

Al-Sah, A.H., Nasserul, Z., 2002. Splenectomy for children with thalassemia. Int. Surg. 87 (4), 269–673.

Al-Sultan, A., Phanasgaonkar, S., Suliman, A., Al-Baqushi, M., Nasrullah, Z., Al-Ali, A., 2011. Spectrum of β-thalassemia mutations in the eastern province of Saudi Arabia. Hemoglobin 35 (2), 125–134.

Asadov, C., Abdulilumov, E., Mammadova, T., Gafarova, S., Guliyeva, Y., 2017. Aliyeva G genotype-phenotype correlations of β-thalassemia mutations in an Azerbajani population. Turk. J. Haematol. 34 (3), 258–263. https://doi.org/10.2474/tjh.2016.0427.

Bandyopadhyay, A., Bandyopadhyay, S., Chowdhury, M.D., Dasgupta, U.B., 1999. Major β-globin gene mutations in Eastern India and their associated haplotypes. Hum. Hered. 49, 232–235.

Bircan, I., Sisli, S., Guven, A., et al., 1993. Hemoglobinopathies in the district of Antalya. Turkey. Pediatr. Hematol. Oncol. 10, 289–291.

Bolaman, Z., Enli, Y., Koseoglu, M., et al., 2001. Prevalence of Beta Thalassemia trait in Denizli. Turkish J. Haem. 18, 85–88.

Cao, A., Gossens, M., Pirastu, M., 1989. β-Thalassemia mutations in Mediterranean populations. Br. J. Haematol. 71, 309–312.

Chang, J.G., Lu, J.M., Huang, J.M., Chen, J.T., Liu, H.J., Chang, C.P., 1995. Rapid diagnosis of beta-thalassemia by mutagenically separated polymerase chain reaction (MS-PCR) and its application to prenatal diagnosis. Br. J. Haematol. 91, 602–607.

Chia-Cheng, H., Shieh-Uan Chen, Shin-Yu Lin, Mei-Ya Fang, Li-Jung Chang, Yi-Yi Tsai, Li-Ting Lin, Yu-Shih Yang, Chien-Nan Lee, Yi-Ning Su, 2010. Preimplantation genetic diagnosis of beta-thalassemia using real-time polymerase chain reaction with fluorescence resonance energy transfer hybridization probes. Anal. Biochem. 400(1), 69–77.

Chomyn, K.P., 2018. Economic burden of transfusion dependent thalassemia. Indian J. Pediatr. 85 (5), 329–330. https://doi.org/10.1007/s12098-018-2642-z.

Colosimo, A., Gatta, V., Guida, V., Leodori, E., Foglietta, E., Rinaldi, S., Cappabianca, M. F., Amato, A., Stuppia, L., Dallapiccola, B., 2011. Application of MLPA assay to characterize unsolved α-globin gene rearrangements. Blood Cells Mol. Dis. 46 (2), 139–144.

Darwish, H.M., El-Khatib, F.F., Ayesh, S., 2005. Spectrum of β-thalassemia mutations among thalassemia patients in the West Bank region of Palestine. Hemoglobin 29 (2), 119–132.

Derkachshandeh-Peykar, P., Akhavan-Niaki, H., Tamaddoni, A., 2007. Distribution of β-thalassemia mutations in the northern provinces of Iran. Hemoglobin 31 (3), 191–196.

El-Hazmi, M.A., 1982. Haemoglobin disorders: a pattern for thalassaemia and other haemoglobinopathies in Arabia. Acta Haematol. 68, 43–51.

El-Hazmi, M.A., al-Swailem, A.R., Warsy, A.S., 1995. Molecular defects in beta-thalassaeas in the population of Saudi Arabia. Hum. Hered. 45, 278–285.

El-Hazmi, M.A., Warsy, A.S., 1996. β-thalassemia mutations in the Arabian Gulf. Hemoglobin 20, 10–14.

El-Hazmi, M.A., Warsy, A.S., 1999. Appraisal of sickle-cell and thalassaemia genes in Saudi Arabia. East Mediterr Health J. 5, 1147–1153.

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References

Abd-Elsalam, K.A., 2003. Bioinformatic tools and guideline for PCR primer design. African J. Biotechnol. 2(5), 91–95.

Abdulrahman, W.A., Jamaludin, N.B., Kham, S.K., Tan, J.A., 1996. The spectrum of beta-thalassaemia mutations in Malays in Singapore and Kedah. Southeast Asian J. Trop. Med. Public Health 27, 164–168.

Addour, N.B., Zaidi, N., Beldjoud, C., Belkhellil, M., 2011. Molecular heterogeneity of beta-thalassemia in Algeria. In: 12th International Conference on Thalassaemia and Other Haemoglobinopathies. 14th Tif Conference for Patients and Parents. May 11 (14), 43.

AfraziAli, A., Karimi, M., Dehghani, S.J., Dehbozorgian, J., Asadzade, R., Amiri, A., Sahgotaleslam, N., Sotodegan, F., Morshed, N., Razzaqi, S., 2011. Beta-thalassemia screening through prenatal diagnosis in southern Iran. Arab J. Basic Appl. Sci. 18, 393–396.

Al-Salem, A.M., Warsy, A.S., 1995. Molecular defects in beta-thalassaemia trait in Saudi Arabia. East Mediterr Health J. 5, 1147–1153.

Al-Allawi, N.A., Jubrael, J.M., Hughson, M., 2006. Molecular characterization of beta-thalassemia in the Dehok region of Iraq. Hemoglobin 30 (4), 479–486.

El-Hazmi, M.A., al-Swailem, A.R., Warsy, A.S., 1995. Molecular defects in beta-thalassaemia trait in Saudi Arabia. Acta Haematol. 68, 43–51.

El-Hazmi, M.A., al-Swailem, A.R., Warsy, A.S., 1995. Molecular defects in beta-thalassaemia trait in Saudi Arabia. Acta Haematol. 68, 43–51.

El-Hazmi, M.A., Warsy, A.S., 1999. Appraisal of sickle-cell and thalassaemia genes in Saudi Arabia. East Mediterr Health J. 5, 1147–1153.
