Prognosis of an Inherited Beta Globin Deficiency in Sickle Cell Anemic Iraqi Population

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Samples of 500 patients suffering from Sickle Cell Disease (SCD) were collected from Ibn Al-Baladi hospital and subjected for blood analysis. Most patients showed an elevated level of HbF, HbA2, iron and eosinophils. Two primers were designed to amplify two regions of α-globin gene, the first targeting the site from which gene expression begins, and the other is targeting the coding region Dgn83. Results showed the presence of a common pathogenic mutation of Arab countries at HBB, LOC107133510, LOC110006319 with phenotype MIM 603903, while changes were detected at Dgn83 with no attribution to SCD. It is concluded that such mutation is the main cause of SCD in Arab countries with specific phenotype that differ from other countries around the world.

Keyword: SCD, Blood disorder, Beta Globin, Mutation.

Sickle cell disease (SCD) is a progeny transferred blood illness that influence the function of red blood cells. People with this disease produce an altered form of Hb called HbS. Sickle cell conditions are transferred from parental line in the same way as blood typing. Sickle cell disease manifests due to a unique nucleotide change in the Beta-globin gene, which is, a replacement of glutamic acid with valine at position 6 of the beta chain (Imoru et al., 2011). Altered forms of beta-globin will deform red blood cells into a sickle form that die quickly, leading to anemia (Christyand Benjamin, 2001). The non heterozygous HbS is designated as sickle cell anaemia (SCA) is considered as the widely dominant form of SCD, the rate is variable regarding the country where it is detected (Imoru et al., 2011 ; Ansong et al., 2013). The next predominant occurrence of SCD is the synergistic trait of HbS and HbC referred to as HbSC, which mostly found in West of Africa (Piel et al., 2017). SCD is resembled by protean indicators ranging from acute generalized pain to early onset stroke, leg ulcers and the risk of early deaths from organ failure. As a an outcome of the effect of HbF, clinical symptoms do not begin until the median to 2nd part of the first year after birth when this has predominantly become to adult haemoglobin (Akinsheye et al., 2011). People with SCD are more susceptible to severe infections, especially from certain types of bacteria, causing pneumonia, meningitis, septicaemia or bone infections. Children with SCD have a high ratio of getting severe, life-threatening infections (Tubman and Makani 2017). Haemoglobin electrophoresis can be used as screening method that can identify the phenotype of SCA but is not a reliable method for the determination of genotype in infants less than 6 months because of high...
levels of fetal haemoglobin (haemoglobin F) that circulating from birth which is the predominant haemoglobin at this age (Emuejevoke et al., 2018). Plenty of work has been associated with the search for genetic influence comes from beta gene cluster region that might alter globin gene expression and, thus, reveals the clinical diversity of sickle cell disease (SCD). Beta gene sequence alteration has presented independently in countries of the world with different genetic backgrounds. Beta genes are found in unbalance with at least five different defined haplotypes in the Beta-globin gene cluster (Praneeta et al., 2015; Hira et al., 2016). Distribution of these haplotypes is determined geographically, specificity and high homogeneity in each region (Liang et al., 2014).

Ethnic DNA might control the expression of the beta globin gene and consequently play a role in the determined variations of the phenotypic expression of SCD (Akinsheye et al., 2011).

Gene therapy as early studies may be a possible treatment for sickle cell anaemia. The technique is based on stem cells and gene therapy; instead of using embryonic stem cells. The aim is to transform a patient’s blood cells into pluripotent stem cells and replace the defective portion of the gene (Ribeil et al., 2017).

A number of new sickle cell therapeutic methods are on the horizon; the promise of combination therapy is no longer a far-fetched aspiration. It is therefore timely to commission such a review on newborn sickle cell screening not just for European countries which of course face the migration challenge, but also other countries (Inusa et al., 2018).

MATERIALS AND METHODS

Patients and sampling

Total 500 blood samples were collected patients from suffering Sickle cell anemia (SCA) from March 2018 to October 2018, at Ibn Al-Baladi Hospital (Baghdad) with (100) blood samples served as control group. The ages of patients and control group (Healthy) were 10–40 years.

Hematological analysis

Complete blood count was carried out using a counter model (Apex Bio Medicals, Germany). Sickle cell hemoglobin was quantitated after elution from a microcolumn of diethylamineethyl cellulose (DE 52) resin as described earlier (Habibzadeh et al., 1999). Fetal hemoglobin quantitation was performed by alkaline denaturation procedure (Betke et al., 1959).

DNA extraction

DNA was obtained from blood samples using the Reliaprep genomic DNA MiniPrep System from Geneaid /Korea according to company instructions, concatenation and purity of the extracted DNA was measured using nanodrop (Techne /UK).

Primers used for DNA amplification

Beta- globin exons were amplified using the following primers designed in this study table (1).

PCR amplification Protocols

The DNA extraction from patients and control blood was amplified by polymerase chain reaction (PCR) using (specific primers) designed specifically to target Beta- Globin gene at specific locations using the following program: Initial denaturation at (95°C) for (5) minutes, with (35) cycles of Denaturation at (94°C) for (30) seconds, Annealing as given in Table (1) for (30) seconds , Extension at (72°C) for (30) seconds, followed by Final Extension at (72°C) for (7) minutes.

DNA sequencing

Amplicon from PCR amplification of Beta- Globin gene regions were sent for sequencing by Macrogen Company / Korea. The sequences of these samples (patients and control) were analyzed using available software on (NCBI) National Center for Biotechnology Information like BLAST of SNPs using available data.

RESULTS

Blood test results were obtained by measuring PCV, Hb, RBC, MCV, MCH and reticulocytes count which are listed in table (2) in comparison with normal values and healthy.

The results show that the MCV and MCH values are reduced in comparison with normal range and control (healthy) since they resemble the ratio between RBCs with PCV and RBCs with Hb respectively that gave clear indication for the presence of hemolysis in RBCs as a result of the disease.
Blood leukocytes in SCA patients blood were investigated for their ratio and abnormality. Results obtained are listed in table (3) showing an increased level in WBCs as a result of continuous blood transfusion.

The same result was found when HbF was studied, since the elevated levels were found due to high production of α2, α2 subunits which, are the main components in the HbF architecture.

Making use of standard measurements in hematologic analysis of patients blood, the type of SCD can be identified either to be homozygous or heterozygous by observing the presence or absence of α– chain in blood samples, level of HbS, HbA, HbA2, and HbF in regard to standard values of these hemoglobins level in healthy people. Table (4) shows the mean percentage of hemoglobin in healthy and patients that were investigated for hemoglobin abnormality. It was concluded that HbF, and HbA2 levels were increased as compared with the normal levels in the healthy, and patient due to the hyper – production of α2, α2 (the subunits of HbF) and α2, α2 (the subunits in HbA) to cope with body demands of oxygen and nutrients as the body grows which cannot be afforded by the low level of HbA in blood. Hence, patients with SCD can be diagnosed by testing HbS, HbA, HbF, and HbA2 levels in blood.

In this study two specific primers were used to detect α– globin (BG) gene in patients and healthy. These primers are BG1, and BG2 that are complementary to a defined region in the

| Primer name | Sequences 3—5 | Product size bp | Annealing temperature °C |
|-------------|----------------|----------------|-------------------------|
| BG 1        | F: GGACCTCTGTCTCTCTCGCT R: GGGACAAGGCTGCAAGCTAT | 296 | 57 |
| BG 2        | F:TGAGAGCTGCTGAGTTGTGT | 435 | 55 |

Table 1. Sequences of primers used in the procedures of the present study with PCR product size. Each one was given the optimum Annealing Temperature

| No. | Red Blood Index | Normal Range | Control (healthy) | Patients |
|-----|----------------|--------------|------------------|----------|
| 1–  | Packed cell volume (PCV) | F: 38 – 45 % M: 40 – 58 % | 43% | 25% |
| 2–  | Hemoglobin (Hb) | F: 12 – 16 g/dl M: 14 – 18 g/dl | 15 g/dl | 11.7 g/dl |
| 3–  | Red blood cells count (RBCs) | F: 3.8 – 5.8 x10¹² M: 3.8 – 5.8 x10¹² | 4.5 x 10¹² | 5.8 x 10¹²/L |
| 4–  | Mean corpuscular volume (MCV) | 82 – 100 fl | 95 fl | 47.3 fl |
| 5–  | Mean corpuscular hemoglobin (MCH) | 27.5 – 33.2 pg | 32 pg | 27 pg |
| 6–  | Reticulocytes | 0.2 – 2 % | 0.4 | 5% |

Table 2. Red blood cells indices in SCA patients and their healthy

| No. | WBC | Normal Range | Control (healthy) | Patient |
|-----|-----|--------------|------------------|---------|
| 1.  | WBC count | 4 – 11 x10⁹ /L | 9 x 10⁹/L | 17 x10⁹/L |
| 2.  | Neutrophile | 40 – 75 % | 65 % | 67 % |
| 3.  | Lymphocyte | 20 – 45 % | 35 % | 20 % |
| 4.  | Monocyte | 2 – 10 % | 2 % | 3 % |
| 5.  | Eosinophiles | 1 – 6 % | 2 % | 10 % |
| 6.  | Basophiles | < 1% | — | — |
| 7.  | Platelet count | 150 – 400 x 10⁹/L | 250 x 10⁹/L | 304 x 10⁹/L |

Table 3. Leukocyte percentage in SCA patients and their healthy
â – globin gene. Results of PCR amplification and electrophoresis of product are shown in figure (1).

The DNA sequencing of the BG gene was taken from blood samples of patients and was compared using the NCBI nucleotide blast as shown in figure (2).

The region flanking the Hemoglobin Subunit Beta Gene (HBBG) resembles multiple sequences that can function as origins of DNA replication. The both endogenously and in ectopic contexts. These origins replicate at the end in many cell types but early in cells that produce hemoglobin, meaning that replication starts in this region may rely on activity of the globin locus control region (LCR). Pathogenic change in DNA sequence at this region may leads to production of abnormal HBB and producing HBS form which SCD to appear. Using primer 1 that was used to amplify this region, we were able to identify a pathogenic mutation similar to that was reported for Arab region SCD as given below.

**Dgn83 coding region**

Primer 2 that was designed to amplify Dgn83 region showed that it has the location of (CACA) motif, at sites “1927, “835, “598, and “543 which is then detected in three Hb A chromosomes. However, Dgn 83 Hbc has an intervening polymorphisms that are identical to Hb A chromosomes.

**DISCUSSION**

Blood disorder (SCD) is a public health threatening disease that influenced millions of people through the many countries. There is an increased value in reticulocytes as a result of continuous blood transfusion and iron precipitation due blood hemolysis in specific body parts like spleen and liver. There is an increase in eosinophiles as a result elevated iron levels. Blood transfusion will make the body more sensitive and adopt more production of eosinophiles. Thus, in many cases the blood should be filtered before it is transfused to patients (Ahmed et al., 2017). There was an elevated level in HbF and HbA₂ which may be explained as an outcome of the increased requirements of the body for oxygen and nutrient demands which are not fully satisfied by

### Table 4. Percentage of hemoglobin types obtained from blood analysis SCA patients and their healthy

| No. | Hemoglobin types | Normal Range% | Control (healthy) | Patients |
|-----|------------------|----------------|-------------------|----------|
| 1-  | HbS              | 0              | 0                 | 15       |
| 2-  | HbF              | 0.5 – 1.5      | 1                 | 16       |
| 3-  | HbA₂             | 1.8 – 3.5      | 2.5               | 7        |
| 4-  | HbA              | 96 – 98        | 96.5              | 90       |

![Fig. 1. PCR analysis of â – globin gene using BG1 and BG2 primers. Lane (1) DNA marker (100 bp). (A) Primer BG1, lanes (2-9) patients. (B) Primer BG2, lanes (2-10) patients](image-url)
Fig. 2. The automated sequencing of BG gene in SCA patients

| Variation type and location | Associated gene | Protein change | Condition | Clinical significance |
|-----------------------------|-----------------|----------------|-----------|-----------------------|
| NM_000518.4(HBB):c.364 G>A (p.Glu122Lys) | HBB, LOC107133510, LOC110006319 | E121K, E122K | Sickle cell-Hemoglobin O Arab disease, Hb SS disease, beta Thalassemia, HEMOGLOBIN O (ARAB), HEMOGLOBIN EGYPT, not provided |

Furthermore, the phenotype-gene Relationships was identify as given in the following details.

| Location | Phenotype          | Phenotype MIM number | Inheritance | Phenotype mapping key | Gene/Locus MIM number |
|----------|--------------------|----------------------|-------------|-----------------------|-----------------------|
| 11p15.4  | Sickle cell anemia | 603903               | AR          | 3                     | HBB 141900            |

the main hemoglobin (HbA) due to the reduction in α – chain formation and / or the truncation or deformation in this protein that results in HbA malfunction. SCD is one of the most serious of the red blood cell diseases, and is caused by an abnormal haemoglobin known as HbS (Ashley-Koch et al., 2000). While normal red blood cells can survive for up to 120 days, sickle cells diminish in just 20 days. The diagnosis of sickle cell anemia relies on the electrophoresis of hemoglobins in hemolysates prepared from the peripheral blood. However, several relatively hemoglobin variants have an electrophoretic mobility identical to that of Hb S on cellulose acetate. Although the diagnosis of SCD is straightforward, that of Hb Sb thalassemia may sometimes be problematic. In Hb Sb thalassemia, there is a preponderance of Hb S with Hb A comprising 5–30 per cent of the total. Hb Sb0 thalassemia produces an electrophoretic pattern that is visually indistinguishable from that.
of sickle cell anemia, but a diagnosis can often be made by the presence of an elevated Hb A2 level and a decreased MCV. However, detailed family history and DNA-based studies may be necessary to make this distinction (Winfred and John, 1999). Genetically, it is a disease caused by replacement of an A-to-T point mutation in the 3-globin gene producing altered hemoglobin S (Hb S), which depletes in the deoxygenated state, thus, causing physical and functional change of erythrocytes. It was described clinically as an inherited blood disorder because of mutations in the beta globin gene, most commonly known as SNP rs334. It is found mostly in African and related populations (Shriner and Rotimi, 2018). SCD results from a nucleotide 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 resembled by chronic haemolysis, recurrent vasoconstriction, rapid infection, organs failure in the body, a periodic pain, abnormal hemoglobin in red blood cells, causing them to turn into the form of solid sickles (Lionne et al., 2012). Chronic haemolysis can lead to different 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 (Abbas et al., 2013).

Despite sickle cell disease (SCD) is considered as homogenic, but its clinical effect is highly heterogeneous. Plenty of the affecting conditions are genetically affected while others are result of environmental factors that come from the region. Considerable number of SCD patients in the Arabian region has the Arab/India haplotype and are represented by elevated Hb F levels which is the first sign of blood disorders (Praneeta et al., 2015). Haemoglobin electrophoresis is a useful diagnosis method that can determine the phenotype of SCA but is not a reliable for the determination of genotype in infants less than 6 months as a result of high levels of fetal haemoglobin (haemoglobin F) that persists from birth which is predominant haemoglobin at this age (Akanni et al., 2013). There is tight and specific relation of Hb F levels and several SNPs in the HBS1L region located on chromosome 6q23 (HBFQTL2; 142470). The relationship of different SNPs in this region were none related to one another, but summation could count for 5% of variance in Hb F levels (Maryam et al., 2020).

CONCLUSIONS

Sickle Cell disease is a lethal disease that is common in Arab countries if the patient is not subjected to treatment. Blood tests show instantly an elevated levels of HbF and HbA2. Most of patients are treated with blood transfusion which eventually will elevate iron level and WBCs count. The main cause of this disease is a pathogenic mutation lies within non coding region of 3-globin gene. This mutation is similar to that found in other Arabian countries like Egypt with gene MIM number 141900.

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