# ABSENCE OF SP1 TRANSCRIPTION FACTOR VARIANT IN 102 IRAQI PATIENTS WITH BETA THALASSEMIA INTERMEDIA

DILAN ALBARAWI, BSc, MSc*
JAFFAR NOURI JAFFAR ALALSAIDISSA, MBChB, MSc, PhD**
NASIR AL-ALLAWI, MBChB, MSc, PhD***

*Submitted 8/5/2017; accepted 30/6/2017*

## ABSTRACT

**Background:** Several genetic mechanisms contribute to the phenotype of β- thalassemia intermedia. Many studies have focused on identifying these mechanisms; however, they did not explain all such cases, leaving a lot of area for further research. Recently a candidate gene (Sp1 transcription factor variant) has been identified as a possible contributor to amelioration of phenotype in β-thalassemia intermedia.

**Subject and Method:** To determine the relative frequency of Sp1 variant and its contribution to amelioration of phenotype in Iraqi patients with β-thalassemia intermedia. A total of 102 molecularly characterized Iraqi patients with β-thalassemia intermedia attending Ibn-Albaladi hereditary anemia center in Baghdad-Iraq, had their records evaluated and their DNA screened for the Sp1 transcription factor variant (R170Q) using amplification refractory mutation systems-polymerase chain reaction.

**Results:** None of the 102 enrolled patients with β-thalassemia intermedia carried this mutation, and all showed the wild type Sp1 (R170Q).

**Conclusions:** The Sp1 transcription factor variant does not appear to contribute to amelioration of the β-thalassemia phenotype in Iraqi enrolled patients. A search for other factors that maybe contributory is warranted.

**Duhok Med J 2017; 11 (1): 1-7.**

**Keywords:** Thalassemia Intermedia, Sp1 variant, ARMS-PCR, Iraq,

Beta thalassemia is an autosomal recessive disorder associated with a spectrum of clinical phenotypes, ranging from transfusion dependent thalassemia major to mostly asymptomatic β-thalassemia minor. In between these two extremes the phenotype of β-thalassemia intermedia lies.[1] A variety of genetic mechanisms may be responsible for the latter phenotype, including inheritance of mild $\beta^+$ mutations, coinheritance of alpha thalassemia, and inheritance of determinants associated with augmented γ-chain production.[2] The latter will lead to increased HbF production (α2γ2) and is linked to three major quantitative trait loci (QTLs) namely: HBG2 , BCL11A and HMIP on chromosomes 11p, 2p, and 6q respectively. However, these QTL explain only 20-50% of HbF variability, [3] leaving a lot of area for further research in other loci for single nucleotide polymorphisms (SNPs) that may contribute. In one report on Iraqi twins with transfusion

* Assistant lecturer, Scientific Research center, College of Science, University of Duhok, Kurdistan Region, Iraq
** Lecture, Department of Pathology, College of Medicine, University of Duhok, Kurdistan Region, Iraq
*** Professor, Department of Pathology and Scientific Research center, College of Medicine, University of Duhok, Kurdistan Region, Iraq
***Correspondence author to:*** *Nasir Al-Allawi, nallawi@yahoo.com, Mobil +9647504551494*

https://doi.org/10.31386/dmj.2017.11.1

independent thalassemia intermedia, several candidate genes that may be contributory were identified.[4] Among these candidate genes a mutation involving the Sp1 gene on chromosome 12 was identified, which leads to an Arginine to glutamic acid at position 170 of the SP1 transcription factor.

Sp1 factor has been identified in the 1980s as a zinc-finger protein with transcriptional activity, which functions by regulating GC rich promoters.[5] Sp1 binding sites have been detected in the promoter regions of several genes, including the γ-globin gene, indicating a direct role of this factor in many regulated processes, including Hemoglobin F production.[6,7]

To investigate the role of Sp1 transcription factor variant in amelioration of phenotype in β-thalassemia, we screened for it in a group of Iraqi patients with β-thalassemia intermedia, who were already extensively studied by one of the authors,[8] with the hope of determining if it has any contribution to HbF variability or phenotype in this cohort.

## PATIENTS AND METHODS

DNA stored at the biobank of the Scientific Research Center at the College of Medicine, University of Duhok for 102 Iraqi patients with β-thalassemia Intermedia and reported by an earlier study from this center was used.[8] The patients were enrolled from the Ibn Albaladi Hereditary Anemia center in Baghdad, Iraq and all had originally signed informed consents, which included having their DNA screened for any mutations that maybe relevant to their thalassemia. Additionally the current study was approved by the ethical committee at the college of Science, University of Duhok, Iraq.

The extracted DNA was amplified based on an Amplification Refractory Mutation System (ARMS) method developed at university of Boston, USA (personal communication). The primers used were SP1F 5' ACCAGCAAGTTCTGAC 3', SP1G1 5'GTGCAAACCAACAGAT 3', SP1A1 5'CAATGATGTTGCCTCC 3' and SP1R 5'TGTGATGATACCAAGC 3'. The amplification mix was prepared by adding to 12.5 µl of 2xTaq Mix (GeneScript-Germany), 7.5 µl dH2O, and 1 µl each of primers at a concentration of 50 pmol/µl , and 1 µl of template DNA. The reaction program was run on an ABI thermocycler (USA) with the following steps: Step 1: $96^0$ C 5 minutes; Step 2 (Cycling for 30 cycles) with $96^0$ C (20s), $58^0$ C (40 s), $72^0$ C (60 s); Step 3: $72^0$ C (10 minutes). The amplicons were then run on a 2 % agarose gel and the results were photo-documented.

## RESULTS

**Patients' characteristics:** The 102 enrolled patients had ages ranging from 3 to 58 years (Median 13 years) and included 62 males and 40 females. The median age at diagnosis was 4 years. Six patients were never transfused, while median age for first transfusion for the remaining patients was 5 years. The median hemoglobin at the time of enrolment was 8.1 g/dl, while HbF% in the 62 patients in which it was available varied widely from 7.1%-98.4%. Moreover, it was found that 21 different β thalassemia mutations were identified and they were arranged in the genotypes of $\beta^+/\beta^+$ in 33,

$\beta^+/\beta^0$ in 17, $\beta^0/\beta^0$ in 47, $\beta^0$/HbE in 2 and β-thal carrier in 3 cases.[8]

**Molecular studies:** Homozygous for Sp1 variant would have a 471 and 207bp bands, those with the wild type 471 and 317 bp bands, while heterozygous will have a 471, 317 and 207 bp. Figure 1 shows gel electrophoresis on 2% agarose gel of a group of the enrolled patients, including a heterozygous control (lane labeled C). It shows that all the samples tested had a wild type pattern (471 and 317 bp), while the control (C) clearly shows a 3 band pattern (471, 317 and 207 bp). All 102 enrolled thalassemia intermedia patients had a wild type pattern and thus none had the Sp1 variant (R170Q).

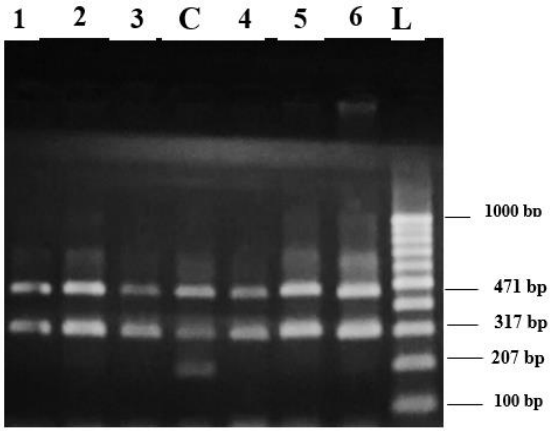


**Figure 1. Agarose gel electrophoresis (2%) on amplicons after ARMS for SP1 variant, showing that cases 1-3 and 4-6 have the wild type genotype, with two bands (471 and 317bp). The Control [C] is heterozygous with 3 bands (207, 317 and 471 bp). Lane L at the right hand side is the 100bp ladder.**

## DISCUSSION

Several factors may ameliorate phenotype in Iraqi patients with β-thalassemia intermedia. The most important are mild β mutations ($\beta^+$), encountered in almost half of patients with thalassemia intermedia included in the current study.[8] While most of the remaining cases had the severe $\beta^0/\beta^0$ genotype.[8] The latter genotype is classically associated with thalassemia major rather than intermedia. Two SNPs at HBG2 (rs7482144) and BCL11A (rs10189857) linked to HbF production, were identified as contributors to amelioration of an otherwise thalassemia major phenotype. However, the study concluded that this did not fully explain this phenotypic variability and the need to search for other contributory factors is needed. In 2013, Jiang and coworkers investigating Iraqi twins who were homozygous for severe frame shift codon 8 (-AA) mutation, and who were virtually asymptomatic, identified several candidate genes that may be associated with globin gene expression, by whole genome sequencing and validated by Sanger DNA sequencing, including Sp1 variant (R170Q).[4] This promising observation on Iraqi patients with thalassemia intermedia triggered the current study on molecularly characterized cohort of patients with this phenotype. However, we were unable to identify any thalassemia intermedia patient with $\beta^0/\beta^0$ or for that matter with any other genotypes, with the Sp1 transcription factor variant among our 102 enrolled patients. Similarly, searching for the Sp1 variant in 22 patients from Iraq, Morocco and Turkey who had Codon 8 (-AA) homozygosity, but were mostly transfusion dependent (severe), failed to identify variant in any.[9] Similar observations were also documented by another study on 41 patients with sickle cell disease and high HbF% and 25 patients with homozygous or compound heterozygous β-thalassemia, none of whom carried this mutation.[10]

The absence Sp1 variant in our thalassemia intermedia patients as well as in other

groups studied earlier, indicates that this variant is quite rare. However, the presence of several binding site for Sp1 on the promoters of the γ- gene on chromosome 11, and the finding that mutations in the promoter region would result in enhanced binding of SP1 factor and in increased HBG gene expression, suggests that it may have a role in amelioration of β-thalassemia phenotype, in patients who carry this mutation.[6,7,11] Though because of its extreme rarity among Iraqis as well as other populations, it role as an important contributor to the thalassemia intermedia phenotype is muted.

In conclusion, it appears that while the current study does not rule out a role for Sp1 transcription factor variant in modulating HbF in particular Iraqi patients with β-thalassemia, however its role, if any, is quite limited most likely because it is a rare variant in this population. Further studies on other variants are still needed to elucidate possible role in HbF modulation and amelioration of phenotype in thalassemia.

## REFERENCES


1. Camaschella C, Cappellini MD. Thalassemia intermedia. Haematologica. 1995; 80:58–68.
2. Taher A, Vichinsky E, Musallam K, Cappellini MD, Viprakasit V, Weatherall DJ. Guidelines for the management of non-transfusion dependent thalassaemia (NTDT). Thalassemia International Federation, Nicosia, 2013.
3. Thein SL, Menzel S, Lathrop M, Garner C. Control of Fetal Hemoglobin: new sights emerging from genomics and clinical implications. Hum Mol Genet. 2009;18:R216–R223.
4. Jiang Z, Luo H-y, Al-Allawi N, Unal S, Gümrük F, Osorio-Almeida L, et al. Patients Homozygous for Codon 8 (–AA) Frame-Shift $\beta^0$-Thalassemia Mutation with Markedly Increased HbF. Blood. 2013; 122: 3455.
5. Kaczynski J, Cook T, Urrutia R. Sp1- and Kruppel-like transcription factors. Genome Biology. 2003; 4: 206.
6. Gumucio DL, Rood KL, Blanchard-McQuate KL, Gray TA, Saulino A, Collins FS. Interactions of Sp1 with the human γ globin promoter: Binding and transactivation of normal and mutant promoters. Blood. 1991; 78, 1853-1863.
7. Sykes K. & Kaufman R. A naturally occurring γ-globin gene mutation enhances SP1 binding activity. Mol Cell Biol. 1990; 10, 95-102.
8. Al-Allawi NA, Puehringer H, Raheem RA, Oberkanins C. Genetic Modifiers in β-Thalassemia Intermedia: A Study on 102 Iraqi Arab Patients. Genet Test Mol Biomarkers. 2015;19 (5):242-7.
9. Jiang Z, Luo HY, Huang S, Farrell JJ, Davis L, Théberge R, et al. The genetic basis of asymptomatic codon 8 frame-shift (HBB:c25_ 26delAA) β (0) -thalassaemia homozygotes. Brit J Haematol. 2016; 172(6): 958-65.

10. Jiang Z, Luo HY, Farrell JJ, Zhang Z, Schulz VP, Albarawi D, et al. A variant Sp1 (R218Q) transcription factor might enhance HbF expression in $\beta^0$-thalassaemia homozygotes. Brit J Haematol. 2017; In Press.

11. Ronchi, A., Nicolis, S., Santoro, C., Ottolenghi, S. Increased Sp1 binding mediates erythroid-specific overexpression of a mutated (HPFH) γ-globin promoter. Nucleic Acids Res. 1989;17, 10231-10234.

https://doi.org/10.31386/dmj.2017.11.1

## ثوختة

فاکتةرێ وةرطیرانێ یێ جوراوجور ل جینێ SP1 یێ بةرزةبوو ل 102 نةخوشیَن عیراقي ییِن توشبووی ب نةخوشیا تةلاسیمیا B ذ جورێ مام ناوةند

ثێشةکی: طةلةك هوکارێَن بوماوةیی هاریکاربوینة ذ بوَ دةست نیشانکرنا فینوتایبا نةخوشیا تةلاسیمیا ناظنجی ذ جورێ بیَتا. طةلةك ظةکولینا کار لسةر دةست نیشانکرنا میکانیزما ظێ نةخوشیا ناظبری کریة, لێ شروظةکرنا هةمی فاکتةرێَن طریدای ب ظێ نةخوشییێ ب شیَوةیةکی ئاشکرا نةهاتینة دیارکرن و رێ هاتیة ظةکرن بوَ لیَکولین لسةر بهیَنة ئةنجامدان.

ریَکیَن ظةکولینێ: دةرئینانا ریَذا دوبارةبوونێ بوَ(SP1) و روولێ وی بوَ باشتکرنا فینوتایبا نةخوشیَن توشبووی ب نةخوشیا تةلاسیمیا ناظنجی ذ جورێ بیَتا. 102 نموونة هاتنة وةرطرتن ذ هاولاتییَن ئیراقی ئةویَن توشبووی ب نةخوشیا تةلاسیمیا ناظنجی ذ جورێ بیَتا و ئاشکراکرنا ریذا فاکتةرێ طورای SP1 (R170Q) ب ریَکا ب کارئینانا میکانیزما کهورینا جینی ب کةرمیێ بوَ مةزنکرنا کارلیَکا بولیمیراز یا دیف ئیك دا PCR.

ئةنجام: د ئةنجامادا دیاربوو کو هیَج نةخوشیَن توشبووی ب نةخوشیا تةلاسیمیا ناظنجی ذ جورێ بیَتا هةلطرێ جینی بازدای (mutant) نینن و هةمی نموونة بریتی بوون ذ جورێ کیظی (wild) .

دةرئةنجام: فاکتةرێ طورای SP1 هیض رولةکێ طاریطةر نةبوو بوَ باشترکرنا فینوتایبا ئةظێ نةخوشیا ناظبري. ویا ثێتظیة ظةکولینیَن زیَدةتر بهیَنة ئةنجامدان لسةر فاکتةرێَن بةشدار د ظێ نةخوشییێ دا.

# الخلاصة


## غياب طفره عامل النسخ SP1 بين 102 مريضا عراقيا بانيميا البحر المتوسط الوسطى من الفئة بيتا ثلاسيميا

**الخلفية والأهداف:** ساهمت العديد من الاليات في النمط الظاهري لمرضى فقر دم البحر المتوسط الوسطى من الفئة بيتا وقد ركزت العديد من الدراسات على تشخيص هذه الاليات على الرغم انها لم توضح جميع الحالات تاركة العديد من الحقول للدراسات المستقبلية. تقدير التكرار النسبي للمتغير SP1 ومساهمته في تحسين النمط الظاهري بين العراقيين المصابين بفقر الدم البحر المتوسط الوسطى ذات الفئة بيتا.

**طرق البحث:** تم اختبار عينات مؤلفة من 102 عراقيين حاملي مرض فقر الدم البحر المتوسط الوسطى من الفئة بيتا والكشف عن وجود نسخة العامل المتغير SP1 (R170Q) باستخدام نظم التظاعف الحراري – التفاعل التسلسلي البوليمرايزي(PCR) .

**النتائج:** بينت النتائج عدم وجود اي من المرضى المصابين بمرض فقر الدم البحر المتوسط الوسطى من الفئة بيتا حاملين هذه الطفرة وان جميع العينات اظهرت وجود النوع البري (wild).

**الاستنتاجات:** ان العامل المتغير SP1 لم يظهر اي مساهمة في تحسين النمط الظاهري لحالات فقر الدم البحر المتوسط الوسطى من الفئة بيتا , ويتوجب باجراء بحوث اخرى على عوامل اخرى التي قد تساهم في ذلك.


https://doi.org/10.31386/dmj.2017.11.1