

Case Report

Co-inheritance $\alpha\alpha$ anti 3.7 triplication with hemoglobin D/ β^0 thalassemia: A case report from South west of Iran

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Hemoglobin D [Hb D] is the second most common hemoglobin variant in South west of Iran. It places in second position after hemoglobin S. So far up to present, from the genetic point of view, all cases of Hb D are hemoglobin Punjab. Hb D, in all forms, heterozygote, homozygote and compound heterozygote presents with mild anemia or completely asymptomatic that may be discovered accidentally. There was a case presentation of a child with genotype of Hb D/ β^0 thalassemia co-inherited with $\alpha\alpha$ anti 3.7 triplication and phenotype of moderate to severe anemia similar to thalassemia intermediae.

Key words: Hb D-Punjab, $\alpha\alpha$ anti 3.7 triplication, South west of Iran.

INTRODUCTION

A 6-year old boy referred to Shafa hospital with anemia. He is the first child of an Arabian couple with first cousin consanguinity. His father is a 32 years old man with laboratory findings compatible with β thalassemia minor, moreover, his mother with 30 year-old presents with laboratory data identical to Hb D trait (Table 1).

At first visit, the child appears with pallor and breathlessness. He was admitted at hospital for advanced evaluation. In initial physical examination, the positive findings were: Weight: 20 kg, height: 120 cm, subconjunctivae pallor, normal face bone, pulse rate: 120/min, respiratory rate: 20/min, spleen and liver were palpable 7 and 2 cm below costal margin, respectively.

The first cell blood count and hemoglobin electrophoresis of patient and his parents were depicted in Table 1. The patient was transfused 250 ml isogroup packed blood cell based on the initial hemoglobin. He has received blood transfusion every 3 - 6 months since 1.5-year-old. The goal of transfusion strategy program, was the pre- transfusion and steady state hemoglobin 8 and

10 gr/dl, respectively.

MATERIALS AND METHODS

Initially, before blood transfusion 2 ml blood sample was drawn and collected in EDTA for cell blood count and Hb electrophoresis from patient and his parents individually.

Hematological parameters were determined by using an automated Coulter Cell Counter [sysmex k1000], hemoglobin electrophoresis on cellulose acetate at $\text{pH} = 8.6$. Genomic DNA from the proband was extracted before regular blood transfusion and processed by routine salting out method. Alpha and beta globin *gene* was analyzed by using reverse dot blot kit as manufacture instruction (Vienna Lab Co.).

In addition, the whole beta globin *gene* was amplified by two primer pairs (primer pair 5- AGGTACGGCTGTCATCACTTAGA-3 and 5- TTCCAAATAGTAATGTACTAGGCA-3 for exons 1 and 2 and primer pair 5- TCTCTTTCTTTCAGGGCAATAATG-3 and 5- CTTTATGTTTTAAATGCACTGACC-3 for exon 3), which generated products of 970 and 626 base pair length, respectively. The PCR products were directly sequenced by automated sequencer (ABI 3770).

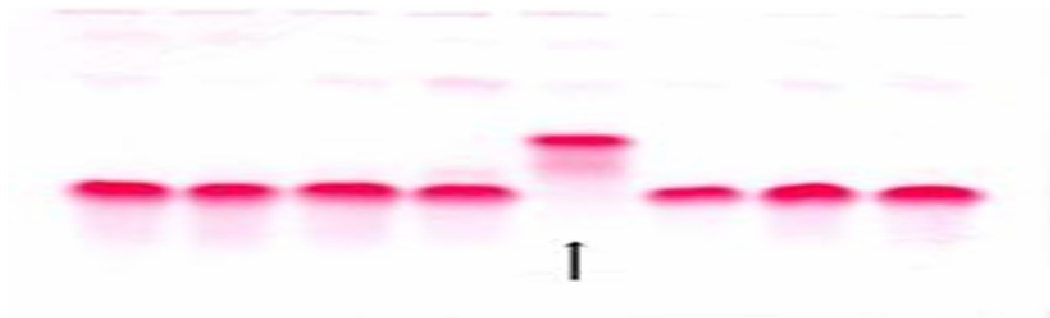
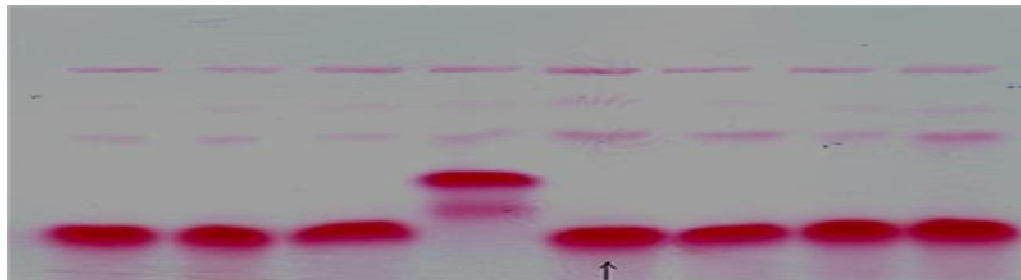
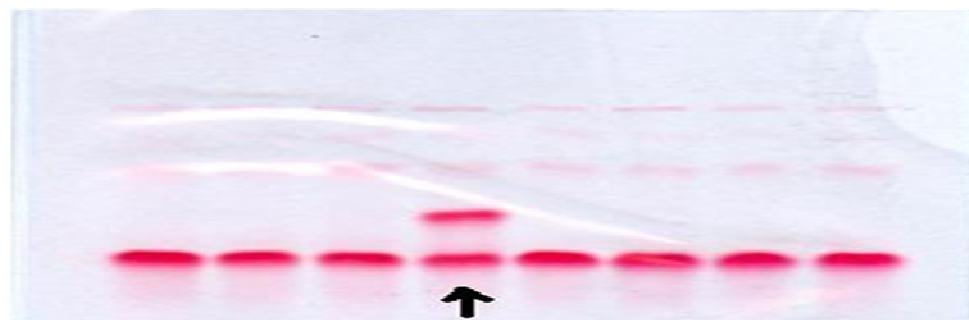
RESULTS

Hematologic parameters and hemoglobin electrophoresis of patient and his parents are shown in Table 1. The

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Table 1. Hematologic parameters and Hb electrophoresis of patient and his parents.

HbD %	HbF %	HbA2 %	HbA1 %	Reticulocyte %	Ferritin (µg/L)	RDW %	MCHC (g/dL)	MCH (pg)	MCV (fL)	HCT %	Hb (g/dL)	RBC Count (*10 ¹² /L)	Age (years)	Patient/parents
-	0.1	4.4	95.5	0.5	115	14.9	28.8	18.8	65.4	40.3	11.6	6.1	32	Father
45.8	0.1	2.2	51.9	0.5	33	12	33.5	28.2	84.3	39.1	13.1	4.6	30	Mother
97.4	0.1	2.5	0	8.2	256	22.8	29.9	17.8	59.5	23.4	7	3.9	6	Son

**Figures 1.** Hemoglobin electrophoresis results from the patient.**Figures 2.** Hemoglobin electrophoresis results from the patient's parents (father).**Figures 3.** Hemoglobin electrophoresis results from the patient's parents (mother).

hemoglobin electrophoresis of patient and his parents illustrated in Figures 1 - 3. Reverse dot blot results of the proband showed anti 3.7-triplication for alpha globin

gene (Figure 4) and IVS-1-5 mutation in a heterozygous manner for the beta globin gene (Figure 5). Direct sequencing of the beta globin gene confirmed the

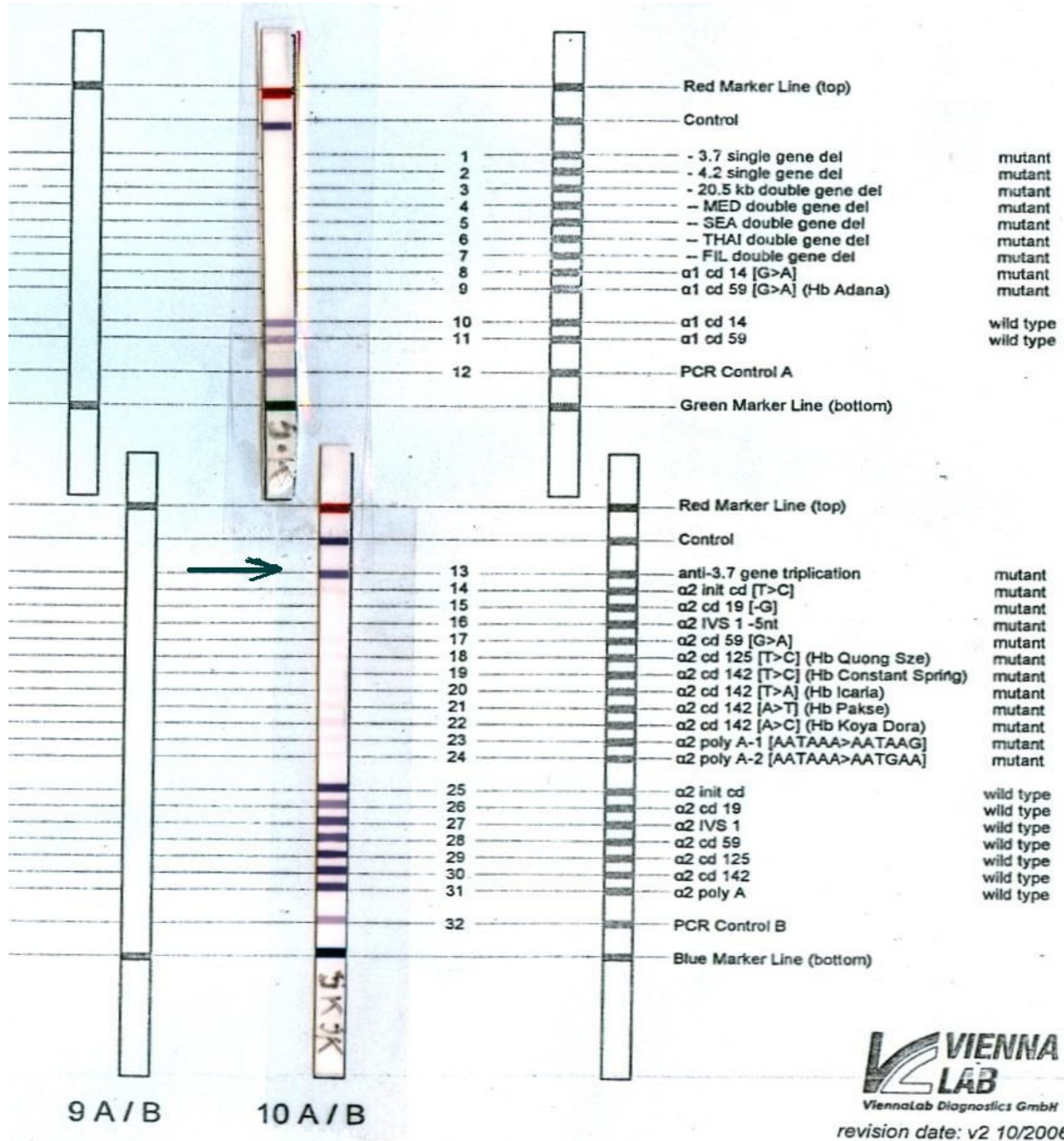


Figure 4. Results of reverse dot blot (RDB) show aaa anti 3.7 triplication in the alpha globin gene.

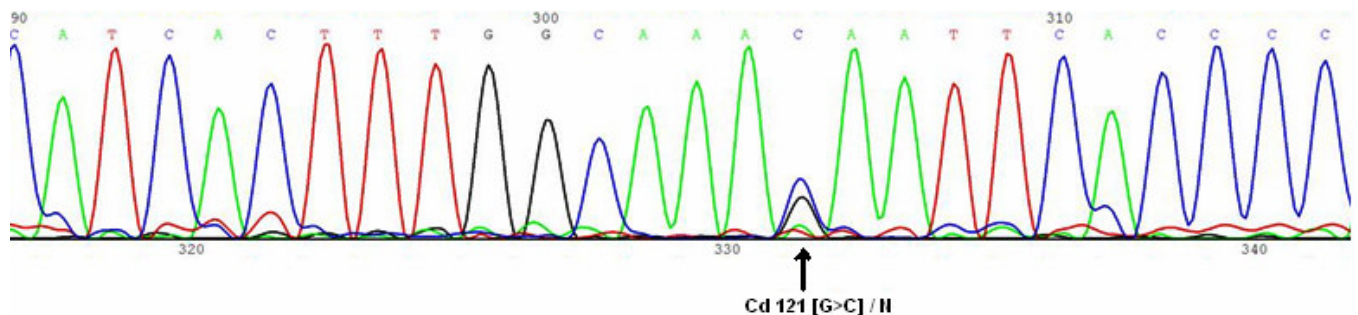
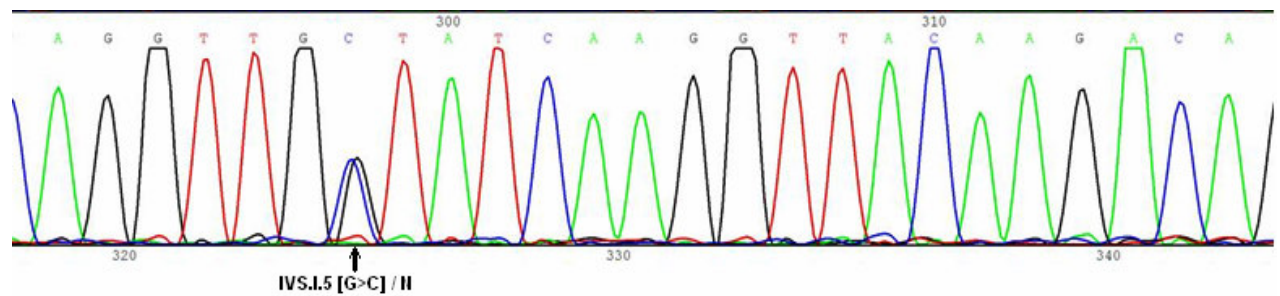
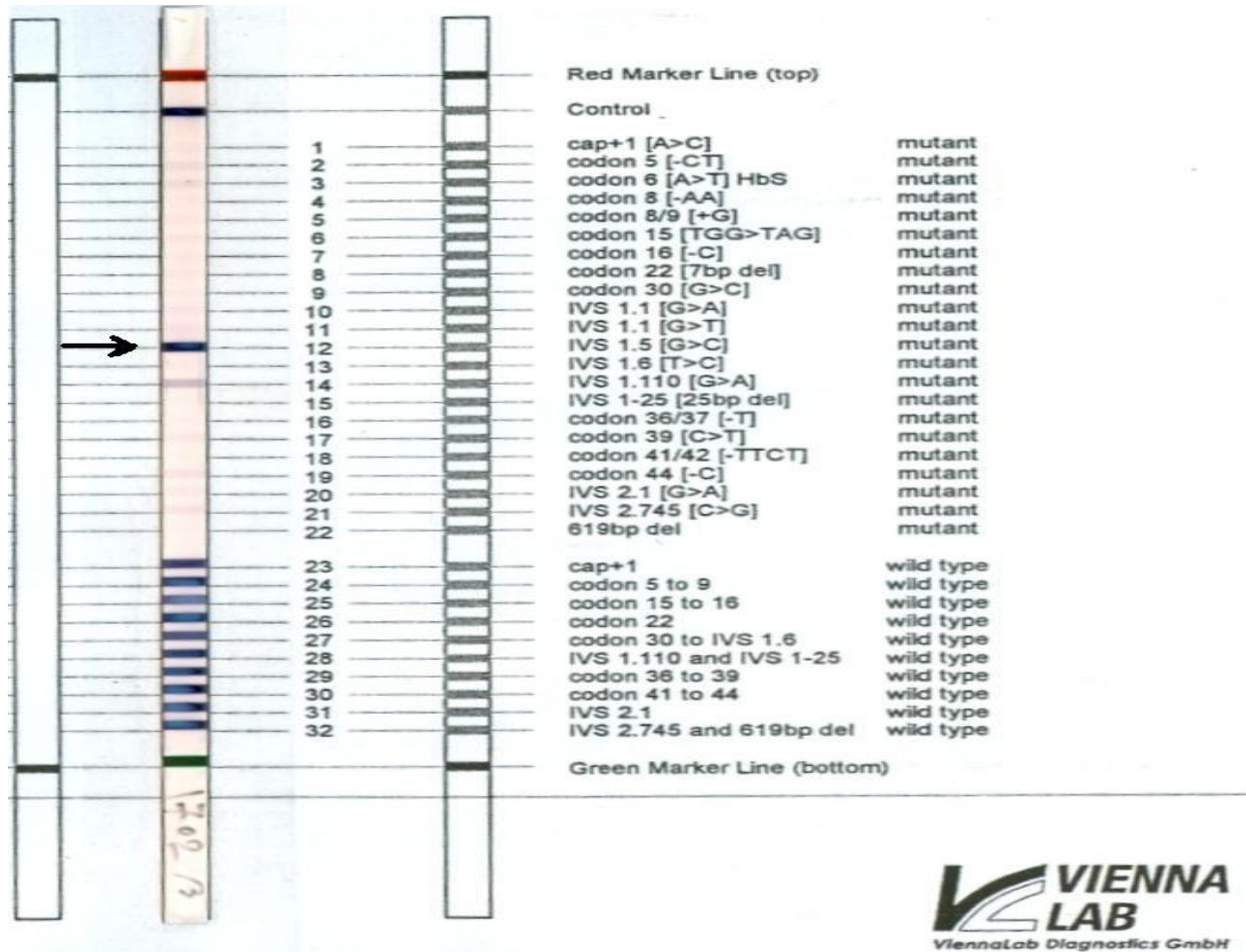
mentioned nucleotide change in which a second change in codon 121 (GAA>CAA). Sequencing results are shown as autoradiogram in Figure 6.

DISCUSSION

In Iran, pre marital genetic counseling program has started since 1996 (Samavat, 2004). Initially, the aim of that strategy was to decrease the new occurrence of beta

thalassemia major. Then, incorporated with the other hemoglobinopaties, such as, HbS homozygous and Hb S/β thalassemia (Zandian et al., 2009).

Hb D [PUNJAB], β I2I Glu-->Gln [GAA-->CAA] was the third hemoglobin variant to be discovered (Itano, 1951). Originally, termed Hb D Los Angeles, it was first described in a family of mixed English, Irish, and American, Indian ancestry (Sturgeon et al., 1955). Hb D [Punjab] has subsequently been well documented in several different widely separated ethnic groups (Baglioni,



Figures 5 - 6. Sequencing of the beta globin gene revealed two mutations in the patient: IVS-1-5 and cd121 (HbD).

1962; Wasi et al., 1968; Ramot et al., 1969).

Hemoglobin D is the second most common hemoglobin variant in south west Iran (Zandian et al., 2009). In Iran, Hb D is predominantly, Hb D Punjab, and a rare case of Hb D Iran (beta 22 Glu-->Gln) has been reported (Zandian et al., 2009; Rahbar, 1973; Rahimi et al., 2006). Up to present, all hemoglobin D in this region of Iran is hemoglobin Punjab. Hb D- Punjab is an abnormal hemoglobin with an amino acid substitution of glutamine for glutamic acid at codon 121 of the beta-globin *gene* [beta 121 [GH4] Glu-->Gln [GAA-->CAA] [also known as Hb D-Los Angeles, D-North Carolina, D-Portugal, D-Chicago and Oak Ridge]. Hemoglobin D even in homozygote and/or compound heterozygote form is asymptomatic or presents with mild anemia (Atalay et al., 2007).

This is a history of a child of 6-year old with genotype of triple mutations as hemoglobin D, Beta thalassemia and $\alpha\alpha\alpha$ anti 3.7 triplications and phenotype of similar to thalassemia intermedia. Among hemoglobin variants, Hemoglobin S and D were frequently found in Iranians in the heterozygous and the homozygous state, and in association with beta-thalassemia (Rahbar, 1973).

Hb D has a normal stability and normal or slightly increased oxygen affinity. It has no deleterious effect on paired β chain and otherwise has a mild effect on the defective β chain. Thus, the interaction of Hb D trait and β -thalassemia trait usually accompanies mild syndrome like thalassemia minor. Hb D heterozygote and homozygote generally have normal hemoglobin values and red cell indices (Atalay et al., 2007; Schnee et al., 1990; Saiki et al., 1988; Perea et al., 1999).

Whereas, Compound heterozygous state of Hb D-Punjab with β^0 thalassemia is not common and it is characterized by a mild-to-moderate hemolytic anemia. Hematological picture of Hb D/ β^0 thalassemia indicates a marked hypochromia and microcytosis and significant elevation of Hb D-Punjab without Hb A₁ (Kirk et al., 1999; Adekile et al., 1996).

Comparable cases with different presentation reported in the literature. Several of them present here. Hynes and Lehmann reported a Persian girl with slight anemia suffering from Hb D/ β^0 thalassemia. That was presumably the first document of Hb D/ β^0 thalassemia in medical literature (Hynes and Lehmann 1956). Sukumaran et al. (1960) reported 4 Indian cases of Hb D/thalassemia. They showed that two of them were non anemic (Sukumaran et al. 1960). Schneider and associates encountered a woman of English, Scotch, and Irish ancestry with a microcytic, hypochromic anemia having a hematocrit value of 28%. Hemoglobin electrophoresis and chromatography revealed 81.7% Hb D [Punjab], 8% HbA, 5.3% HbA₂, and 5% Hb F, indicating heterozygosity for the structural abnormality and a β^+ -thalassemia (Schneider, 1968).

An Indian child with thalassaemic-like red cells, 21% Hb F and 79 %Hb D, and severe anemia [Hematocrit value

23%] was reported by Jam et al., 1970. Ramot et al. 1969, reported a boy of Bulgarian-Jewish ancestry with Hb D - β -thalassemia and moderate anemia. Oldrini et al., 1973 described an Italian woman with 96.5% Hb D, 3.5% HbA₂, and a hematocrit of 32%. The father was a carrier of Hb D, while the mother appeared to have thalassemia minor. In 1976 RF Rieder reported a 23-year old man of Greek-Italian ancestry with mild anemia was found to be heterozygous for Hb D [Punjab] β 121 Glu → Gln and β -thalassemia. (Ronald, 1976).

Three years ago, Zoreh Rahimi et al, 2006, introduced two sons with Hb D-Punjab/ β^0 -thalassemia IVS II.1 [G→A] from west Iran. This report demonstrated that, Hb D-Punjab to be a benign structural variant of Hb, which, in combination with β^0 -thalassemia, produces a minor β -thalassemia intermedia like picture with moderate anemia in the presence of significantly elevated Hb F. (Rahimi et al., 2006). Ahmed et al. (2001), revealed two cases of Hb D/ β^0 thalassemia for the first time among Saudi Arabs. The two patients were diagnosed as having chronic hemolytic anemia of moderate severity and the occasional need for blood transfusions. Genetic analysis enabled the detection of compound heterozygosity for the Hb D-Los Angeles [Punjab]/and β^0 thalassemia with mutations as stop W15X [codon 15 TGG→TGA].

Ayhan et al. (1974), from Turkey reported a case of Hb D and alpha thalassemia with severe anemia. Theodoridou et al. (2009) reported a Greek man of Hb D/ β^0 thalassemia with Hb level 13.9 g/dl and history of two occasions of blood donation. The first observation of double heterozygote disorders of hemoglobin D [Hb D] and beta-thalassemia was described by Piechowiak H in a German family (Piechowiak et al., 1985). Comparable with another cases reported in the literature that are mentioned below:

One Canadian, Wong and Mam (1980), three Portuguese, Sousa et al. (1983), one Englishman, Worthington and Lehmann (1985), one Spanish, Casals et al. (1986), three Kuwaiti Arabs, Adekile et al. (1996), one Indian, Ropero et al. (1996) and one Russian, Troitskaia et al. (1998);

In conclusion, this report demonstrates that, the co-inheritance of $\alpha\alpha\alpha$ anti 3.7 triplication in the propositus is responsible for conferring a deteriorative effect on the benign clinical presentation of Hb D-Punjab/ β^0 thalassemia. The frequency of $\alpha\alpha\alpha$ anti 3.7 allele triplication ranges from 0.008 -0.058.(Sukumaran, 1960; Jam, 1970). Hb D has a softening effect on the β^0 allele. Thus, combination of Hb D/ β^0 presents with a benign nature, and $\alpha\alpha\alpha$ anti 3.7 triplication exaggerates the imbalances α/β ratio that produced in mild degree in Hb D/ β^0 thalassemia.

Unpaired alpha chain excess causes an over hemolysis and shortened red blood cell life span. For this reason, we suggest that the molecular analysis is necessary to make a better assessment for pre-marital couples with Hb D and β thalassemia minor. The patient is now on a

continuous folic acid supplementation and occasional blood transfusion.

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