Interaction of - α 3.7, β Thalassemia Mutation IVS 1-5 and HbD Punjab in a Family: A Case Report

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Abstract Hemoglobin D exist in four form; HbD trait, HbD-thalassemia, HbD sickle cell and HbD homozygous. HbD trait and HbD homozygous generally asymptomatic condition but when HbD co-inherit with thalassemia and sickle cell anemia, produces clinically significant conditions like chronic hemolytic anemia. Here we present a case of HbD Punjab with α 3.7 kb deletion and IVS-1-5 β -thalassemia across a family. Diagnosis of HbD patient was performed by high performance liquid chromatography and complete blood count was measured by automated cell analyzer. Molecular study for common alpha deletions done by Gap-PCR while beta thalassemia mutation identified by ARMS-PCR. Case was clinically significant due to the inheritance of HbD/ β^+ thalassemia genotype. Thus observed case behaved like thalassemia intermedia due to co-existence of α 3.7 deletions with IVS 1-5 β -thalassemia mutation in HbD Punjab patient.

Keywords HbD Punjab · GAP-PCR · HPLC · ARMS-PCR · IVS 1-5

Introduction

HbD Punjab [β 121(GH4)GI-Gln, GAA-CAA] occurs with greatest prevalence (2%) in Sikhs in Punjab, India, whereas Gujarat, the province in the west from where the case was reported, has a prevalence rate of 1% [1]. Another report showed the average gene frequency of HbD was 0.86% with a higher frequency of 3.6% seen in Punjab followed by Jammu and Kashmir (3.3%) and Uttar Pradesh (2.3%) [2]. It is also found sporadically in Blacks and Europeans, the latter usually coming from countries that have had close associations with India in the past [3]. Biochemically, HbD occurs in four forms: heterozygous HbD trait, HbD-thalassemia, Hb S-D disease, and the rare homozygous HbD disease, which is associated with a clinical disorder similar to, but less severe than, sickle cell anemia [4]. HbD has been reported in association with hematological malignancy such as leukemia and Hodgkin's lymphoma [5]. Thus our aim was to present this report to show the clinical and hematological profile of HbD patient due to interaction of the alpha deletions and β -thalassemia mutation and their inheritance across the family.

Case History

1-year-old female child from Uttar pradesh came to hematology OPD AIIMS with severe pneumonia, pallor associated with fever. There was no history of blood transfusion. Physical examination revealed pallor of the skin. The liver and spleen were not enlarged and there was no skeletal deformity. Cardiovascular and nervous systems were normal. She had one brother who showed only mild clinical findings i. e., anemic; while her parents were asymptomatic.

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Material and Method

Blood samples were collected in 5 ml vacutainer containing EDTA as an anticoagulant. Complete blood count and red cell indices were measured by automated Analyzer (SYSMEX K-4500, Kobe Japan) Giemsa-stained peripheral blood smear were examined for red cell morphology. Quantitative assessment of hemoglobin, HbF, HbA, HbA₂, HbD was performed by HPLC (Bio-Rad-Variant™ Bio Rad, CA, USA). Hemogram and HPLC Chromatogram of patient presented in Figs. 1 and 2, respectively. DNA isolation was done by phenol chloroform method while alpha thalassemia deletions and beta mutations investigated by GAP and ARMS-PCR, respectively according to published literatures [6–9].

Result and Discussion

The patient family was had mother, father and one brother. The HPLC report and molecular study showed; patient was

compound heterozygous for HbD/ β^+ thal, mother was HbD Punjab trait while father was heterozygous β/β^+ thalassemia genotype and brother was heterozygous for HbD Punjab. Patient's retic count was 4.2%; RBC microcytic hypochromic and peripheral smear showed target cell and spherocyte while serum bilirubin was 0.7. Mother retic count was 0.4%, RBC normocytic normochromic. Father retic count was 2.4% RBC normocytic normochromic and Brother retic count was 0.5%, RBC normocytic normochromic, peripheral smear showed target cell and spherocyte. All hematological investigations were summarized in Table 1.

Molecular characterization for four common α thalassemia deletions and five common β thalassemia mutation were performed and result showed the mother was heterozygous for α 3.7 kb deletions while father was α 3.7 kb heterozygous and also positive for IVS1-5. The patient was homozygous for α 3.7 kb deletion and positive for IVS 1-5 β thalassemia mutation while brother was only homozygous for α 3.7 kb deletion. (Gel picture of α 3.7 kb and IVS 1-5 presented in Figs. 3 and 4, respectively). HbD Punjab has

Fig. 1 Complete blood count of patient

Fig. 2 HPLC chromatogram of patient **Table 1** Hematological data of family

Age Sex	Patient 1 Year Female	Mother 30 Year Female	Father 34 Year Male	Brother 4 Year Male
HbA	49.6	46.4	83.6	48.4
HbA2	3.0	3.0	6	1.8
HbF	0	0	1.2	0.9
HbD	38.2	42.9	0	37.8
WBC	6.28	7.3	8.23	5.07
RBC	3.8	4.26	4.82	3.29
HGB	11.4	11.6	12.3	9.8
HCT	37.6	27.8	39.4	32.4
MCV	78.3	65.3	81.7	98.5
MCH	23.8	20.2	25.5	29.8
MCHC	30.3	30.9	31.2	30.2
PLT	197	187	294	215

been described in both the heterozygous and homozygous states as well as in combination with HbS or β -thalassemia. Simple heterozygous and homozygous individuals with HbD Punjab are asymptomatic, whereas association with

HbS is characterized by a mild to moderate hemolytic anemia [10]. HbD- β thalassemia is generally a very mild condition. However, HbSD disease may manifest with variable clinical features [11]. HbS and HbD are one of the

Fig. 3 A 3.7 kb inheritance across the family. (Lane 1A, 2A, 3A, 4A mutant set and lane 1B, 2B, 3B, 4B normal set. Lane 1AB patient homozygous, 2AB mother heterozygous, 3AB brother homozygous and 4AB father heterozygous. Primer A+ C (A) normal and A+ B(B) mutant are amplified in separate tubes because their product has the same size of 1.8 kb - Baysal et al. [6])

Fig. 4 IVS 1-5 inheritance across the family

commonly encountered Hb variants worldwide [12]. The major concern for ruling out HbD- beta zero thalassemia is that homozygous HbD disease causes mild hemolytic anemia, but co-inheritance of beta zero thalassemia seems to give deleterious effects on the presentation of HbD disease, leading to chronic hemolytic anemia of moderate severity [13]. In our case; due to co-inheritance of α 3.7 kb deletion and IVS 1-5 β thalassemia mutation in hemoglobin D patient, clinically and hematological feature suggestive like thalassemia intermedia, however, the frequency of this combination is unknown. In conclusion the hemoglobin D Punjab patients clinically behaved like thalassemia intermedia due to inheritance of beta thalassemia mutations with alpha deletions together.

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