ORIGINAL ARTICLE

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Hemoglobin D and Coinheritance with Hb S, β-thalassemia

Hemoglobin D ve Hb D'nin β-Talasemi ve Hb S ile Birlikte Kalıtımı

ABSTRACT

Objective:

Hb D-Punjab (also known as D-Los Angeles) is a recessively inherited variant of hemoglobin. It is one of the most common hemoglobin variants worldwide. In this study, we aimed to evaluate the hematological features of our homozygous and heterozygous Hb D patients and patients with compound heterozygosity for Hb D and other hemoglobinopathies in terms of clinical and laboratory findings.

Material and Methods:

In this study, cases with Hb D-Punjab have been identified in both the heterozygous and homozygous states, as well as coinheritance with Hb S or β-thalassemia. We presented the clinical and laboratory characteristics of 18 cases (Hb D/D (n=2), Hb D/β-thalassemia (n=3) and Hb S/D (n=3) and Hb D traits (n=10)).

Results:

As a result of the study, it was observed that Hb D was asymptomatic in both heterozygous and homozygous forms. Hb D/β thalassemia cases showed mild microcytic and hypochromic anemia, but they were clinically normal. Compound heterozygosity for Hb S/D showed moderate hemolytic anemia, but a severe clinical picture with painful crises, just like sickle cell patients.

Conclusion:

Hemoglobin D-Punjab, which is asymptomatic even in homozygous condition, can cause a variety of clinical pictures from mild to severe when inherited in combination with other hemoglobinopathies.

Key Words:

Hemoglobin D, Hemoglobin S, Beta thalassemia, Hemoglobinopathy

ÖZET

Amaç:

Hb D-Punjab (D-Los Angeles olarak da bilinir), çekinik olarak kalıtılan bir hemoglobin çeşididir. Dünya çapında en yaygın hemoglobin varyantlarından biridir. Bu çalışmada homozigot ve heterozigot Hb D hastalarımız ve Hb D ile diğer hemoglobinopatilerin bileşik heterozigotluğu olan hastalarımızın hematolojik özelliklerini klinik ve laboratuvar bulguları açısından değerlendirmeyi amaçladık.

Gereç ve Yöntemler:

Bu çalışmada, Hb D-Punjab'lı vakalar hem heterozigot hem de homozigot durumlarda ve ayrıca Hb S veya β-talasemi ile birlikte kalıtımda tanımlanmıştır. On sekiz olgunun (Hb D/D (n=2), Hb D/β-talasemi (n=3) ve Hb S/D (n=3) ve Hb D (n=10)) klinik ve laboratuvar özelliklerini sunduk.

Bulgular:

Çalışma sonucunda Hb D'nin hem heterozigot hem de homozigot formda asemptomatik olduğu gözlendi. Hb D/β talasemi olguları hafif mikrositik ve hipokromik anemi gösterdi ancak klinik olarak normaldi. Hb S/D bileşik heterozigotluğu, orta derecede hemolitik anemi gösterdi, ancak orak hücre hastaları gibi ağrılı krizlerle şiddetli bir klinik tablo gösterdi.

Sonuç:

Homozigot durumda bile asemptomatik olan Hemoglobin D-Punjab, diğer hemoglobinopatilerle birlikte kalıtıldığında hafiften şiddetliye kadar çeşitli klinik tablolara neden olabilir.

Anahtar Kelimeler:

Hemoglobin D, Hemoglobin S, Beta talasemi, Hemoglobinopati

INTRODUCTION

Hb D-Punjab (β121(GH4) Glu→Gln) is an inherited hemoglobin variant. Hemoglobin D (Hb D) differs from Hb A as a result of the structural difference of beta-globin chain at position 121, where glutamine replaces glutamic acid (1). It is also known as hemoglobin D-Los Angeles. Hemoglobin D is the third most common hemoglobinopathy in the worldwide after Hb S and Hb C and it is quite prevalent in Pakistan, Northwest India, China and Middle Eastern countries (2-4). Although hemoglobin D generally remains hematologically silent in heterozygous form, its compound heterozygosity with other hemoglobinopathies can cause mild to severe clinical symptoms (5-8). Homozygosity for Hb D (Hb DD) is observed infrequently. Homozygous Hb D cases are usually asymptomatic, but in rare cases, mild hemolytic anemia may be observed. (2, 9). Compound heterozygosity for Hb D-Punjab and β-thalassemia (Both Hb D/β+-thal and Hb D/β0-thal) has been reported in different studies (5, 10-14). In the literature, compound heterozygosity for Hb D-Punjab and β-thalassemia have been reported in a limited number of cases (12-15). In those studies, it has been shown that patients with Hb D/β-thal had mild to moderate anemia. Combined heterozygosity of Hb D Punjab and Hb S (Hb S/D) shows clinical similarity to homozygous sickle cell (HbSS) disease. In addition to cases with mild and moderate symptoms, cases with severe clinical findings such as acute chest syndrome (ACS), have been reported (16, 17). In this study, the clinical and laboratory features of Hb DD, Hb S/D, Hb D/β-thal cases, followed in pediatric hematology-oncology department, are presented. To clarify the hematological picture of Hb D, erythrocyte parameters of Hb D carriers were also evaluated in the study.

MATERIAL and METHODS

We report 8 Hb D cases in combination with different hemoglobinopathies and 10 Hb D traits, diagnosed at Akdeniz University Hospital, Pediatric Hematology-Oncology Department. The data was reviewed retrospectively from patient charts and electronic records. Clinical characteristics and laboratory data, such as erythrocyte parameters in complete blood count analysis, Hb electrophoresis and mutation analysis, were recorded. Hb D diagnoses were made using high-performance liquid chromatography (HPLC) and confirmed by molecular analysis.

RESULTS

In our study, we present the data of 18 cases, carrying at least one Hb D mutation. Nine of these cases were male and 9 were female. The ages of patients at admission to our center, were taken as a basis, and ranged between 5 months and 32 years. We present a total of 8 cases, who had homozygous Hb D and combined heterozygosity of Hb D with other hemoglobinopathies. The mean age of these cases (6 males and 2 females) was 14.43 years (6 months-29 years). Laboratory data for these cases is shown in Table I. We also present the data for 10 Hb D carriers (3 males, 7 females) (Table II). The mean age of these cases was found as 18.3 years.

Cases with Hb DD

We report two cases (Case 1 and Case 2) with homozygote Hb D disease. Both patients had no clinical problems. Laboratory findings of the patients are shown in Table I. Case 2 had normal hemoglobin and MCV levels, but Case 1 had mild microcytic anemia. Since his ferritin value was lower than the normal levels, in the case, anemia was thought to be caused by iron deficiency and oral iron medication was administered. After iron treatment, the Hb level of the patient increased to normal level. However, despite the increase the MCV value of this patient did not reach the normal level (MCV value increased from 61.4 to 71.9). Since MCV value did not improve as a result of iron treatment in this patient, hemoglobin electrophoresis was performed and Hb D variant was detected. Case 2 was diagnosed as a result of the mandatory premarital hemoglobinopathy screening program. As seen in Table I, this patient did not have anemia and low MCV.

Table I. The clinical and laboratory characteristics of hemoglobin D variants

Patient	Diagnosis	Sex	Age (year)	Hb (g/dL)	MCV (fL)	MCH	HbA_1 (%)	HbA_2 (%)	HbF (%)	HbD (%)	HbS (%)
Case 1	HbD/HbD	M	1	10.9*	61.4	21	6.4	1.8	1.7	90.1	-
Case 2	HbD/HbD	M	25	14	80	27.8	6.1	2.2	0.3	91.4	-
Case 3	HbD/β^+-Thalasemia (IVS.1.110)	F	12	10.4	58.4	19.6	12	3.5	2.6	81.9	-
Case 4	HbD/β^+-Thalasemia (IVS.1.110)	F	17	11.1	*61.9	20.4	5.6	3	1.4	77.4	-
Case 5	HbD/β^0 Thalasemia (FSC44)	M	5 months	9.1**	54.1	18.8	9.3	2	25.4	63.3	-
Case 6	HbD/S	M	29	8.5	66.8	20.1	2.6	4.8	3.8	47.4	38.3
Case 7	HbD/S	M	16	9.8	82.7	26.9	2.8	3.7	5.4	41.7	40.6
Case 8	HbD/S	M	15	8.0	90.7	30.9	2.8	2.7	12.8	43	36.6

Hb: Hemoglobin; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hb: *After iron medication the Hb level of the patient increased to 12.0 and the MCV value increased to 71.9 (reference level 80-102 fL)**Hb level of the patient is 10.1g/dL currently

Table II. The clinical and laboratory characteristics of the Hb D traits

Patients	Sex	Age (year)	Hb (g/dL)	MCV (fL)	MCH (pg/)	RBC ($\times 10^6$/mm^3)	RDW (%)	HbA_1 (%)	HbA_2 (%)	HbF (%)	HbD (%)
Case 9	M	2	12	73.9	24.7	4.87	13.7	48.8	2.2	4.2	44.8
Case 10	M	6	12.3	92.8	31.8	3.88	16.4	56.4	1.6	1.5	40.5
Case 11	F	30	13.8	91.7	32.9	4.19	13.4	58	1.9	0.5	39.6
Case 12	F	32	13.7	89.6	29.5	4.64	13.9	61.4	2.5	0.9	35.2
Case 13	F	26	13.8	86.9	29.2	4.73	12.9	59.8	2.4	0.5	37.3
Case 14	F	9	13.6	85	28.6	4.16	13.6	58.6	2.7	0.8	37.9
Case 15	F	17	10.9	74	26.8	4.07	12.4	55.3	1.7	1.4	35.4
Case16	M	18	15.4	79.5	29	5.31	12.3	51.5	1.2	0.2	38.1
Case 17	F	15	17	84.5	27.6	6.14	14.6	64.2	2.3	0.8	32.7
Case 18	F	28	12.8	81.6	27.6	4.64	11.2	61.3	1.7	0.3	36.7

M:Male; F: Female; Hb: Hemoglobin; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hb; RBC: Red Blood Cell; RDW: Red Blood Cell Distribution Width

Cases with Hb D/β-thal

There are three Hb D/β-thal cases, followed in our center (Table I). All of the cases had mild microcytic anemia and had no history of transfusion. β thalassemia mutation (IVS I.110 (G>A) was found in two sisters (Case 3 and Case 4) and the male patient (Case 5) had a β mutation (Fsc 44 (-C)). Since beta thalassemia carriage was detected in the mother of the siblings during examinations for the etiology of anemia, they were evaluated for screening purposes when Case 3 was twelve years old and Case 4 was five years old. In complete blood counts ferritin values of the cases were within normal limits and MCV values were significantly low. The diagnoses of the cases, deemed as Hb D/β+-thal using HPLC, were also confirmed by mutation analysis.

Since a routine complete blood count of Case 5 at five months of age revealed hypochromic microcytic anemia and ferritin value was within normal limits, HPLC test was performed and the patient was diagnosed with Hb D/β-thal. He has been regularly using 5 mg folic acid supplement.

All patients took intermittent iron supplements due to iron deficiency.

Cases with Hb S/D

In this study, we report the clinical and hematological findings for three cases, two of whom were siblings (Case 6, Case 7 and Case 8).

Case 6: The patient, who had been examined with the complaint of pallor at the age of seven months, had hepatosplenomegaly. The patient had a familial history of sickle cell disease and patient's mother and father were 2nd degree consanguineous. Tests, performed on the patient himself revealed Hb S: 80.6%, Hb A2: 1.2%, Hb F: 18% values and the sickling test was positive. Mutation analysis could not be performed. When the patient's Hb value dropped < 7 g/dL, erythrocyte suspension (ES) transfusion was required. The patient, who had a history of vaso-occlusive crisis and salmonella osteomyelitis, was followed up with hydroxyurea (Hydrea) and when necessary, ES replacement. A HPLC test, performed 9 years after the diagnosis, showed an Hb D band and it was discovered that the patient had Hb S/D. At the age of 10 years, avascular necrosis of the femoral head had developed. He had been using hydroxyurea for a long time and he needed transfusions 2-3 times a year. The patient had been admitted to the hospital due to recurrent painful crises and received symptomatic treatment for pain relief and hydration.

Case 7: The patient, whose mother was an Hb S carrier and father was an Hb D carrier, had been diagnosed with Hb S/D when she was three months old. Due to hepatosplenomegaly, the patient had undergone splenectomy at six years of age. Despite an appropriate dose of hydroxyurea treatment, the patient showed a vaso-occlusive crisis that required hospitalization twice a week, acute chest syndrome, requiring hospitalization once a month and a history of transfusion requirement at irregular intervals. Allogeneic hematopoietic stem-cell transplantation (HSCT) was performed from an HLA-matched sibling donor at 16 years of age. The patient was being followed up in disease-free status with complete chimerism.

Case 8: Like his older brother (Case 7) this case had also applied to our center for HSCT. The case was diagnosed with Hb S/D during the prenatal period in another health institution. When he applied to our center, he had been hospitalized more than 20 times due to painful crises in the previous year. He also had complaint of acute chest pain, during hydroxyurea use. The patient, who needed transfusions twice a year, had bone pain almost every day. Unrelated donor screening was performed for the patient, who did not have an HLA-matched relative donor, and HSCT was performed from a 9/10 HLA-matched unrelated donor in 2018. On the 17th day post-transplant, GIS and liver GVHD were seen in the patient. Six months after the stem cell transplantation, the patient died due to Aspergillus pneumonia and multi-organ failure.

Cases with Hb D trait.

In our study, we showed the data of 10 Hb D carriers with ages ranging between 2 and 32 years. Laboratory findings of the cases with Hb D traits are shown in Table II. Except for one of the individuals with Hb D trait (Case 15), none of them had low Hb or low MCV. None of the cases had clinical symptoms. All of our cases were molecularly confirmed as heterozygous Hb D. In the DNA mutation analysis, Hb D (Glu-Gln) and genotype were as Cod.121 (G-C)/normal in the 3rd region of the β-globin gene.

DISCUSSION

Cases with Hb D/D

Hb D is the third most common abnormal hemoglobin after Hb S and Hb C worldwide. However, in the literature, there are very few publications concerning Homozygous Hb D. The reason for infrequent observation of homozygous Hb D cases could be the inability to reach a diagnosis due to the normal clinical and laboratory values of these individuals. For example, in one study, a 41-year-old woman who had been a blood donor, asymptomatic and having normal blood values, has been shown to have Hb D/D (9). HPLC analysis of individuals with homozygous Hb D-Punjab, show large (over 90%) Hb D and normal Hb F and Hb A2 peaks. On the other hand, low Hb A2 was observed in Case 1 in our study. Hemoglobin, MCV and ferritin values of this patient, were also low. Iron supplement therapy was given to the patient and Hb and MCV values of the patient increased to the normal range. However, since the HPLC test was not repeated after the administration of iron supplementation therapy, it is not known whether there was an increase in Hb A2.

Cases with Hb D/ β-thalassemia

While homozygous Hb D patients are asymptomatic, findings such as moderate anemia and hepatosplenomegaly

can be seen in patients with Hb D/β-thal. Hemoglobin values of all our patients in our study were above 10g/dL. Their clinical conditions were also improving well. Similar to the patients in our study, cases without severe anemia symptoms, showing hematological findings similar to beta thalassemia carriers, have been reported in various publications (5, 11). However, in one study, cases with much lower hemoglobin levels have been reported. Iron deficiency has not been mentioned in connection with the cases, presented in that study. The reason for low hemoglobin values could be an existing iron deficiency anemia. Iron deficiency anemia was also observed in Hb D/β-thal patients in our center and iron supplementation therapy was administered to these patients. In many studies, Hb A2 values were found to be in various ranges (normal or high) (5, 15, 18). A case of mild anemia with the same mutation (IVS-I-110) as our two cases (Case 3 and Case 4), has been reported from Greece and and the patient showed 79% Hb D and 2.3% Hb A2 on HPLC (19). In our study, Hb A2 values of all Hb D/β-thal patients were within the normal range. These different ranges of Hb A2 values show that Hb A2 is not a factor for distinguishing Hb D/β-thal patients from Hb D/Hb D patients. Therefore, mutation analysis is necessary to differentiate these two groups of patients.

Co-inheritance of Hb D and Hb S

In our study, the cases with Hb S/D disease had severe condition with painful crises. Wide clinical variability of Hb S/D-Punjab genotype from mild to severe, has been demonstrated in different studies. Torres, et al,.(16) have reported the data for 12 cases with Hb S/D disease and have reported painful crises in 66.7% and ACS in 16.7% of these patients. However, there were no clinical symptoms in three cases. Rezende, et al., have evaluated eleven patients with Hb S/D and have emphasized that the clinical and laboratory findings of children with Hb S/D-Punjab were very similar to those with Hb SS (20). There are many studies, showing that Hb S/D patients had severe hemolytic anemia, as well as severe vaso-occlusive complications (21-23). In one of these studies, an Hb S/D patient, presenting ACS with pulmonary thromboembolism, has also been reported (22). Although the patients in our study had vaso-occlusive crisis during hydroxyurea treatment, studies conducted on a much larger number of patients have shown that hydroxyurea reduced the incidence of vaso-occlusive crises (17). Because of the large number of life-threatening risk factors in Hb S/D patients, early diagnosis and prophylactic treatment are very important. Giving premarital counseling, prenatal diagnosis or pre-implantation genetic diagnosis may prevent the spread of the disease.

Cases with Hb D trait

In HPLC analysis of Hb D carriers, Hb D values are usually found to be approximately 30% to 40% and lower than Hb A. In our study Hb A2 and Hb F levels were within the normal range but Hb F elevation was observed in one case (Case 9). We contemplated that this high Hb F value could be caused by the patient's young age. In some previous studies, Hb D carriers have been reported to be hematologically silent (24, 25). In a study, it has been asserted that Hb D carriers might not be silent and there could be differences in complete blood count, particularly in terms of RBC and RDW values (26). However, complete blood counts of the patients, included in our study, were found to be within normal values. No increases were observed in RBC values. Low MCV value in Case 9 was within the normal range for his age.

CONCLUSION

Both the heterozygous and homozygous Hb D-Punjab are clinically silent conditions. There are no clinical, hematological, or physiological abnormalities in heterozygous Hb D cases. Mild anemia may be present in homozygous patients. Hb D/Beta thalassemia is also evident with significant microcytosis and mild anemia. However, Hb S/D disease causes severe clinical symptoms. Symptoms of sickle cell anemia, such as painful crises, joint necrosis, and sequestration crises are intensified. The clinical situation, caused by the inheritance of Hb D with another hemoglobinopathy, should be well known and genetic counseling is recommended for families, who plan pregnancy in the future. Prenatal diagnosis is required for couples at risk of having an Hb S/D-Punjab child, but not for couples with a high probability of having a homozygous Hb D-Punjab or Hb D-Punjab/β-thal child.

Ethics Committee Approval:

This research complies with all the relevant national regulations, institutional policies and is in accordance the tenets of the Helsinki Declaration, and has been approved by the Akdeniz University, Faculty of Medicine, Clinical Research Ethics Committee (approval number:26.10.2022-652).

Informed Consent:

All the participants' rights were protected and written informed consents were obtained before the procedures according to the Helsinki Declaration.

Author Contributions:

Design – Z.Ö, F.K., A.K.; Data Collection and Processing – Z.Ö., E.G., A.K., F.K.; Literature Search - Z.Ö., F.K; Writing Manuscript – Z.Ö., F.K.; Critical Review – A.K., E.G.

Conflict of Interest:

The authors have no conflict of interest to declare.

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