ISSN : 0974 - 7427 Volume 6 Issue 3

Trade Science Inc.

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 6(3), 2012 [100-103]

Molecular characterization of hemoglobin D β-thalassemia and clinico- hematological presentation of the patients

Sanjay Pandey[1]*, Sweta Pandey[1], Rahasyamani Mishra[2], Renu Saxena[1]

[1]Department of Haematology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi - 110 029, (INDIA)

[2]Department of Environmental Biology, APS University Rewa, (INDIA)

E-mail: pandeysanjaybt@rediffmail.com

Received: 6th February, 2012 ; Accepted: 6th March, 2012

ABSTRACT

HbDβ conditions occur when the β-thalassemia co-inherits with hemoglobin D. Co-inheritance of alpha and beta thalassemia with HbD show the degree of clinical variability. Here we present the clinical variability of HbDβ$^+$thalassemia and HbDβ^{0}thalassemia patients due to presence of alpha deletions and beta mutations. Patients were diagnosed by HPLC while alpha and beta mutation studies done according to published literatures. Our data show clinical variation of HbDβ patients. They were behaved like thalassaemia intermedia and it was due to co-inheritance of alpha deletion and beta mutation.

KEYWORDS

HbD Punjab;
HPLC;
Heterozygous;
Anemia.

INTRODUCTION

Hemoglobin D (HbD), a hemoglobin variant occurs mainly in north-west India, Pakistan and Iran[1]. HbD first encountered to Itano[2], in 1951; differs structurally from normal hemoglobin A at 121 positions on beta chain, where glutamine replaces glutamic acid[3]. HbD occurs in four forms: heterozygous HbD trait, HbD-thalassemia, HbS-D disease and the rare homozygous HbD disease, which is usually associated with mild hemolytic anemia and mild to moderate splenomegaly[4,5]. Average gene frequency of HbD is 0.86% with a higher frequency of 3.6% seen in Punjab followed by Jammu and Kashmir (3.3%) and Uttar Pradesh (2.3%)[6]. Infants with heterozygous HbD/β-thalassemia may be asymptomatic and have mild to moderate hemolytic anemia depending upon the degree of β-thalassemia affecting the A gene. It usually develops in the first few months of life as the amount of HbF decreases and HbD increases. Those with HbD/β+-thalassemia have some HbA and are more likely to have mild to moderate anemia and a non palpable spleen. Children with HbD/β^o-thalassemia syndrome have no HbA, exhibiting symptomatic anemia with spleenomegaly and may have a moderately severe clinical disorder. Because RBC indices are abnormal in HbD/β-thalassemia, iron deficiency may develop[7]. Thus our aim was to determine the clinical nature of the HbDβ patients due to the co-inheritance of various modulating factors.

MATERIAL AND METHOD

Twelve HbD compound heterozygote (HbDβ) pa-

Regular Paper

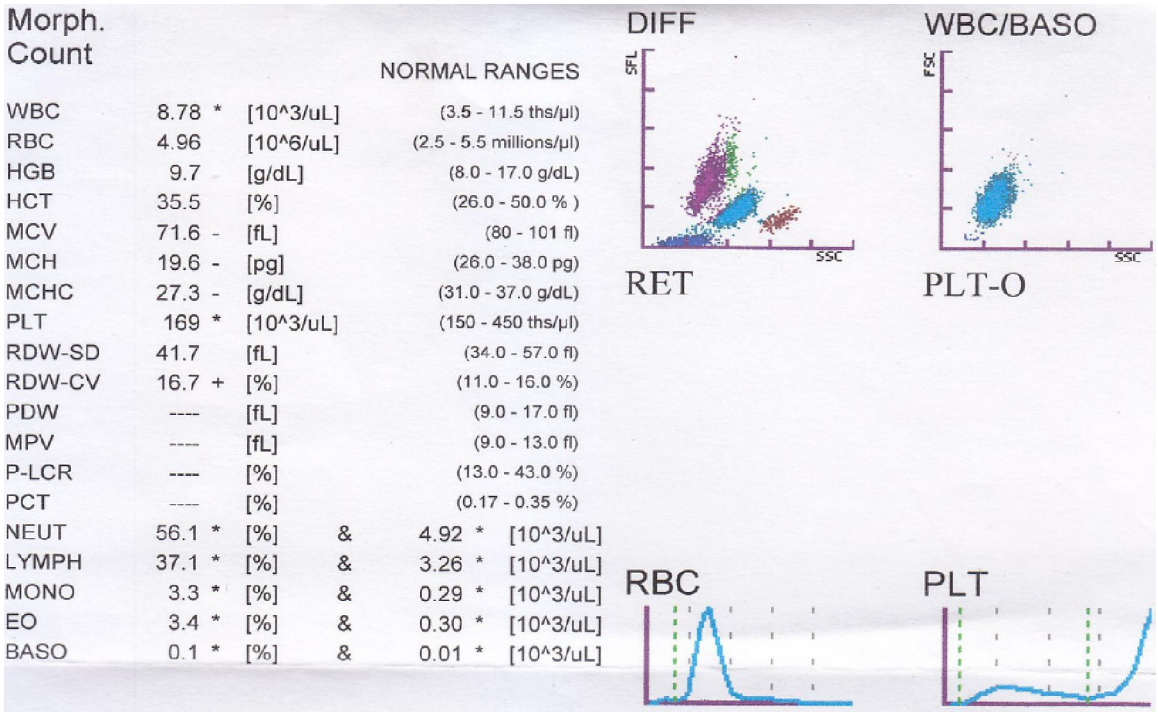

Figure 1 : Hemaogram pattern of HbDβ^{+} patient

tients included in the study, who were attended the outpatient department; All India Institute of Medical Sciences for various complications. Blood samples were collected in 5 ml vacutainer containing EDTA as an anticoagulant after taking their signed consent. This study was approved by institutional ethical committee. Complete blood count and red cell indices were measured by automated analyzer (SYSMEX K-4500, Kobe Japan). Hemogram pattern of patient are given in figure 1. 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). HPLC chromatogram of patient are given in figure 2. Molecular study of four common alpha deletions, five common beta thalassemia mutations and Xmn-1 polymorphism was done according to published literature[8-12].

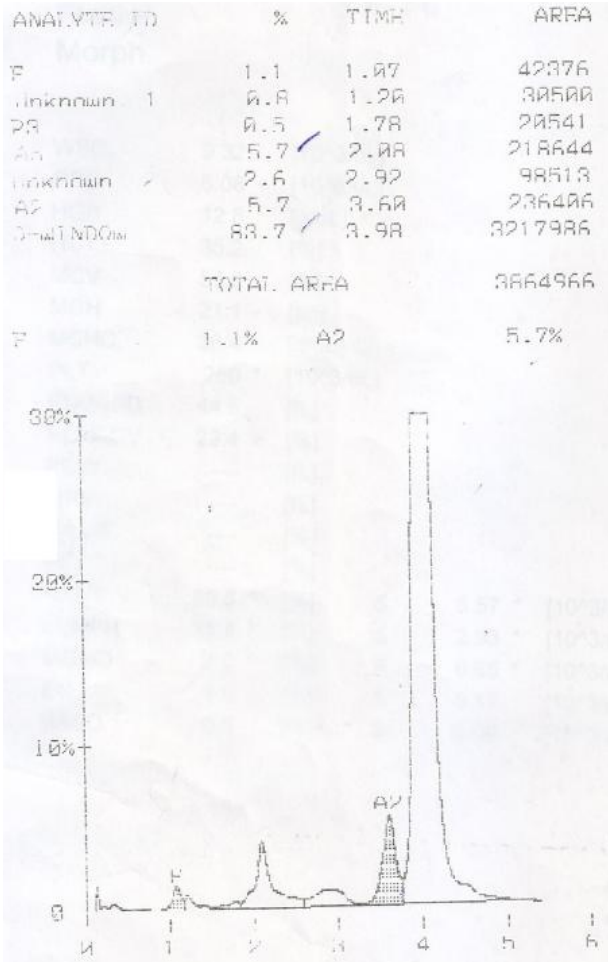

Figure 2 : HPLC chromatogram of HbDβ^{0} patient

RESULT AND DISCUSSION

Total twelve HbDβ patients were included in the study, out of them 9 were HbD/β^{+}(6 male and 3 female with mean age of 14.3±2.7 years) and 3 were HbDβ^{0} (2 male and 1 female with mean age of 13.8±3.5 years). The HbDβ patient's peripheral smears showed micro-

Regular Paper

cytic hypochromic red cells with target cells. The frequency of Weakness, Spleen enlargements, Anemia, pallor was 100% and blood transfusion was 33.34% in HbDβ^0 patients while 33.34, 55.56, 88.89 and 77.78 % in HbDβ$^+$ patients. None of the patients were transfusion dependent in HbDβ$^+$ patients. HbF and HbA2 level were raised; and mean Hb of HbDβ$^+$ (8.3±3.0) and HbDβ^0 (7.3±2.1) were low in patients. Detail clinical and hematological features are given in figure 3 and TABLE 1 respectively. Out of nine HbD/β$^+$ patients; 3 were heterozygous for alpha 3.7^{kb} deletions while one patient was heterozygous for alpha 3.7^{kb} deletion in HbD/β^0 patient (figure 4). Molecular study of beta mutations was determine the 2 patients were IVS 1-5 and one was cd8/9 positive in HbD/β^0 while 5 were IVS 1-5, 2 were cd 8/9 and 2 were 619bp deletion in HbD/β$^+$ patients (figure 5). Xmn1 study was carried for HbD/β$^+$ where 2 patients were heterozygous and one was homozygous while 2 were heterozygous in HbD/β^0 thalassemia (figure 6A and 6B). Heterozygous form of Hb D is clinically silent, but coinheritance of Hb D with Hb S or thalassemia produces clinically significant conditions like sickle cell anemia and chronic hemolytic anemia of moderate severity. In heterozygous condition with co inheritance of thalasemia patient show the degree of clinical variability. HbD has 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 are asymptomatic, where as association with HbS is characterized by a mild to moderate hemolytic anemia[13]. HbD-β thalassemia is generally a very mild condition. However, HbSD disease may manifest with variable clinical features[14]. HbS and HbD are one of the commonly encountered Hb variants worldwide[15]. The major concern for ruling out Hb D- 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 Hb D disease, leading to chronic hemolytic anemia of moderate severity[16]. The association between Hb D and hematological malignancies has also been reported[17]. Patient with hemoglobin D thalassemias hematologic picture belongs to thalassemia trait with moderate hemolytic anemia, intense microcytosis and hypochromia and numerous target cells and Patients presented with mild jaundice, splenomegaly and moderate anaemia[18-21]. In our cases all the patients of HbDβ^0 as well as HbDβ$^+$ were symptomatic had anemia, jaundice spleen enlargement commonly. The symptom of the patients likewise thalassemia intermedia due to the presence of beta tlaalssemia mutations (IVS 1-5, cd 8/9 and 619bp

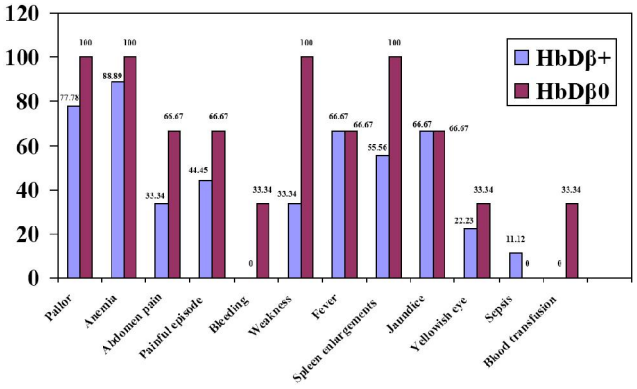

Figure 3 : Comparative clinical feature of HbDβ patients

TABLE 1 : Comprative hematological features of HbDβ-Thal. patients

Hematological Parameters	Mean ± SD	
	HbDβ$^+$ (N=9)	HbDβ^0 (N=3)
Age	14.3 ± 2.7 Years	13.8 ± 3.5 Years
HbA0%	43.2 ± 7.08	40 ± 5.6
HbA2%	3 .54 ± 1.05	4.3 ± 2.7
HbF %	2.1 ± 1.57	2.5 ± 1.7
HbD%	44.54 ± 6.2	45 ± 3.2
WBC Ths/µl	7.9 ± 2.9	6.3 ± 1.5
RBC millions/µl	3.5 ± 1.0	4.2 ± 1.3
HGB g/dl	8.3 ± 3.0	7.3 ± 2.1
HCT%	30.1 ± 7.5	28.3 ± 5.2
MCV fl	73.8 ± 4.1	70.5 ± 3.8
MCH pg	22.5 ± 4.9	25.4 ± 2.1
MCHC g/dl	28.4 ± 3.3	23.8 ± 1.4
PLT Ths/µl	256.4 ± 50.8	165 ± 25.6

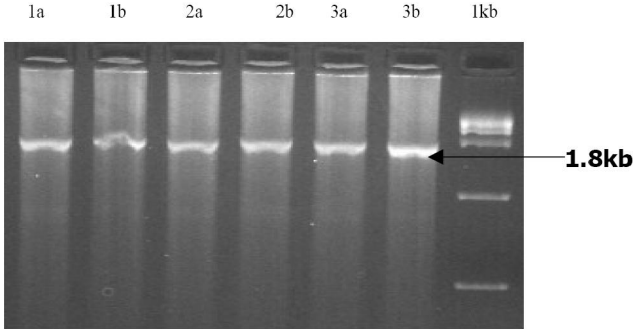

Figure 4 : α$^{3.7}$ heterozygous in HbDβ$^+$ patients

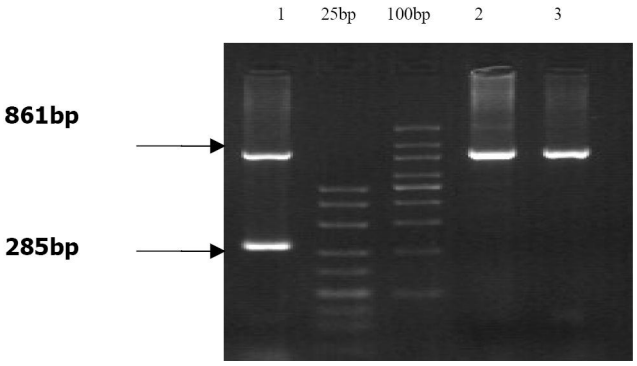

Figure 5 : cd8/9 positive in HbDβ^0 patients (Lane 1)

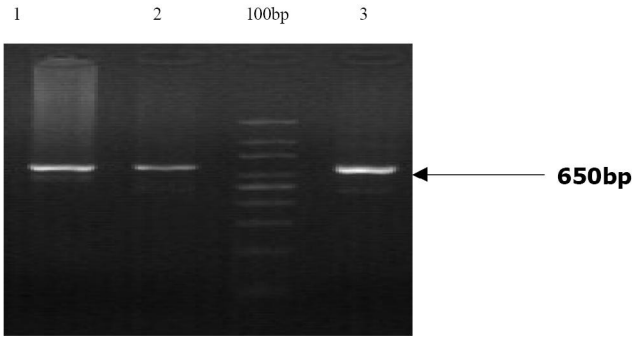

Figure 6A : Check gel for xmn-1 polymorphism

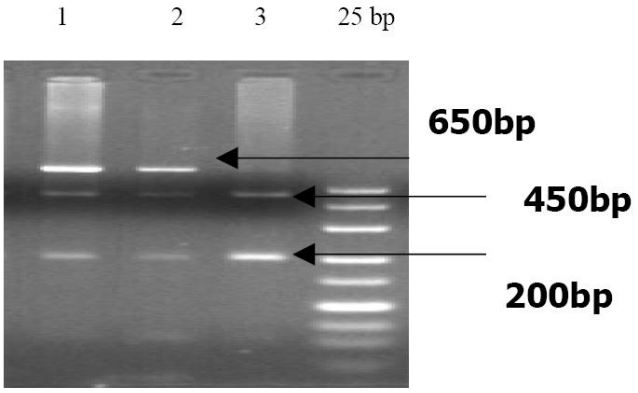

Figure 6B : Xmn-1 polymorphism in HbDβ$^+$ patients (Lane 1,2 are heterozygous and lane 3 is homozygous for Xmn-1 polymorphism)

deletions) and co-inheritance of alpha 3.7 kb deletions in the patients. Co-inheritance of the α deletions and β mutation with hemoglobin D present clinical manifestations from mild to moderate severity.

REFERENCES

[1] F.Firkin, C.Chesterman, D.Penington, B.Rush; Disorders of Hemoglobin Structure and Synthesis. de Gruchi's Clinical Haematology in Medical Practice. 5th Edition, Oxford: Blackwell Science, 137-171 **(1996)**.

[2] H.Itano; Proc.Nat.Acad.Sci.USA, **37**, 775-777 **(1951)**.

[3] C.Baglioni; Biochem.Biophys.Acta, **59**, 437-449 **(1962)**.

[4] J.N.Lukens; The Abnormal Hemoglobins: General Principles. In. G.R.Lee, J.Foerster, J.Lukens, F.Paraskevas, J.P.Greer, G.M.Rodgers, (Eds); Wintrobe's Clinical Hematology. Tenth Edition, Baltimore: Lippincott Williams & Wilkins, 1329-1345 **(1998)**.

[5] S.Ozsoylu; Acta Haematol., **43**, 353-359 **(1970)**.

[6] S.Tyagi, N.Marwaha, V.Parmar, S.Basu; Ind.J. Hematol.Blood.Transf., **18**, 31-32 **(2000)**.

[7] http://health.utah.gov/newbornscreening

[8] E.Baysal, T.H.Huisman; Am.J.Hematol., **46(3)**, 208-213 **(1994)**.

[9] R.V.Shah, S.E.Eunice, S.Baidya, A.Srivastava, M.Chandy; Br.J.Haematol., **123(5)**, 942-947 **(2003)**.

[10] J.G.Chang, L.S.Lee, C.P.Lin, P.H.Chen, C.P.Chen; Blood., **78(3)**, 853-854 **(1991)**.

[11] N.Y.Varawalla, J.M.Old, R.Sarkar, R.Venkatesan, D.J.Weatherall; Br.J.Haematol., **78(2)**, 242-247 **(1991)**.

[12] M.Sutton, E.Bouhassira Eric, L.Ronald, R.L.Nagel; American Journal of Hematology, **32**, 66-69 **(1989)**.

[13] F.J.Perea, M.Casas-Castaneda, A.R.Villalobos-Arambula; Hemoglobin, **23**, 231-237 **(1999)**.

[14] S.El-Kalla, A.R.Mathews; Hemoglobin, **21**, 369-375 **(1997)**.

[15] W.P.Winter; Hemoglobin Variants in Human Populations. CRC Press, Boca Raton, **1&2**, **(1986)**.

[16] M.Ahmed, M.Stuhrmann, L.Bashawri, W.Kuhnau, E.H.El-Harith; Ann Hematol., **80(11)**, 629-633, Nov. **(2001)**.

[17] S.Dash, S.Kumar, R.J.Dash; Hematol., **27(4)**, 305 **(1988)**.

[18] P.Ropero, F.A.González, J.Sánchez, B.Armada, E.Martí, B.Valdés, A.Mora, A.Villegas; Med.Clin. (Barc). **108(10)**, 385-388 **(1997)**.

[19] L.Sousa Uva, A.Fernandes, M.Pilar; Nouv.Rev. Fr.Hematol., **25(6)**, 387-390 **(1983)**.

[20] G.A.Tsistrakis, G.J.Scampardonis, J.P.Clonizakis, L.L.Concouris; Acta Haematol., **54(3)**, 172-179 **(1975)**.

[21] A.D.Adekile, E.G.Kazanetz, J.Y.Leonova, R.Marouf, A.Khmis, T.H.Huisman; J.Pediatr. Hematol.Oncol., **18(2)**, 151-153 **(1996)**.