Phenotypic Effect of α -Globin Gene Numbers on Indian Sickle β -Thalassemia Patients

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Background: Sickle cell β -thalassemia is a compound heterozygous state of β -thalassemia and sickle cell anemia. Patient with these conditions showed mild-to-severe clinical phenotype. **Objectives:** The objective of this study was to evaluate the effects of α -globin gene numbers on the phenotype of sickle cell β -thalassemia patients. **Materials and Methods:** Seventy-five sickle cell β -thalassemia patients were characterized. Clinical, hematological, and molecular characterization was performed in all subjects. Amplified refractory mutation system–polymerase chain reaction was applied for β -thalassemia mutation study while

α -genotyping was conducted by Gap-PCR. **Results:** Highest frequency of IVS1–5 (33 out of 75 patients) β -thalassemia genotype was recorded. Twenty-eight patients were reported with α -globin chain deletion while four had α -triplications (Anti α -3.7kb). Sickle β -thalassemia patients with α -chain deletions ameliorate hematological and clinical variables. **Conclusions:** This study indicates that the coexistence of α -globin chain deletions showed mild phenotype instead of absence of α -chain deletions while the patients with triplication of α -genes express severe phenotype. J. Clin. Lab. Anal. 28:110–113, 2014. © 2014 Wiley Periodicals, Inc.

Key words: α -thalassemia; sickle β -thalassemia; SCD; ARMS-PCR; Gap-PCR

INTRODUCTION

Sickle β -thalassemia condition is caused by a combination of β -thalassemia and sickle cell hemoglobin. Heterozygous sickle trait is completely asymptomatic while compound heterozygous sickle β -thalassemia present mild-to-moderate severity with the coexistence of β -thalassemia mutation. The clinical expression of sickle cell was reported to be variable even within the same population (1, 2). The effect on hematological and biochemical parameter values is influenced by the severity of the disease and the occurrence of the crisis (1, 3). In India, the frequency of the sickle gene reaches as high as 40% especially in the tribal groups (4) whereas the incidence of the β -thalassemia gene is around 3–4% in the general population (5, 6). This heterogeneity is likely to be due to the presence of different β -thalassemia alleles or interaction with modulating genetic factors such as associated α thalassemia and gene for raised HbF production. The coexistence of α -thalassemia with two severe β -thalassemia determinants has been reported to result in a milder condition than β -thalassemia major (7–10). In contrast, tripli-

cation of the α -gene coexistence with a β -thalassemia trait increases the severity of this condition (11–16). However, there have been some conflicting results in the literature (17–20). The modulating effect of α -gene numbers on the phenotype of sickle cell β -thalassemia has not been well characterized in Indian patients. The aim of this study was to determine the clinical and hematological variability under the influence of α -thalassemia genotype in an Indian sickle cell β -thalassemia patient.

MATERIALS AND METHODS

A total of 75 sickle cell β -thalassemia patients were recruited from hematology outpatient department, All

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India Institute of Medical Sciences, New Delhi, India, during the period of 3 years and study was approved by institutional ethical committee. Five milliliters of blood sample was collected from subject after taking their signed consent. Complete blood count and red cell indices were measured by automated cell analyzer (SYSMEX K-4500, Kobe, Japan) while Giemsa-stained peripheral blood smear was examined for red cell morphology. Quantitative assessment of hemoglobin variants and diagnosis of patients were performed by high-performance liquid chromatography (HPLC-Bio-Rad-VariantTM Bio Rad, CA). DNA isolation was done by phenol-chloroform method. Identification of α -deletions and triplication was performed by Gap-PCR while β -thalassemia mutations were identified by amplified refractory mutation system–polymerase chain reaction (ARMS-PCR) according to published literature (21–25). Mean values, standard deviation, and frequency distributions were performed to estimate the hematological and clinical data. A one-way ANOVA test was applied to compare the means of group. P -value < 0.05 was considered statistically significant.

RESULT

The study subjects were categorized into following three groups according to the presence of α -gene numbers. Group 1; 28 patients with α -chain deletion (20 males and 8 females with mean age of 11.14 ± 8.03 years), Group 2; four patients with α -triplication (4 males with mean age of 8 ± 6.05 years), and Group 3; 43 patients without α -chain deletion (33 males and 10 females with mean age of 13.06 ± 8.65 years). Pallor, splenomegaly, gallbladder stone, and jaundice were frequent in α -triplication co-inherited patients. Individual who had α -deletions were reported with higher hemoglobin, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) levels. The IVS1–5(G-C) mutation was found in majority of patients (33 patients) and this was followed by 619 bp (12 patients), IVS I-1 (9 patients), CD8/9 (8 patients), cap site (6 patients), CD41/42 (4 patients), and -88CT (3 patients). Gap-PCR for common deletion mutation showed the highest frequency of α -3.7 heterozygous (16 patients) followed by α -3.7 homozygous (9 patients), and 4.2 heterozygous (3 patients) while four patients had α -triplication. Molecular data are given in Table 1 while comparative hematological and clinical parameters are given in Tables 2 and 3, respectively.

DISCUSSION

Silvestroni and Bianco (26) were the first to describe the compound heterozygosity for the sickle cell and β -thalassemia. Sickle cell β -thalassemia has been reported in different ethnic groups (6). Balagir had reported that

TABLE 1. α -Gene Numbers in Sickle β -Thalassemia Patients

β -genotype	No $N = 75$	α -3.7 het. $N = 16$	α -3.7 hom. $N = 9$	α -4.2 het. $N = 3$	α -trip. $N = 4$
IVS1-5	33	8	3	2	1
IVS-1-1	9	2	1	0	1
CD8/9	8	1	2	0	2
CD41/42	4	1	1	0	0
619 bp del	12	2	1	1	0
Cap site	6	0	1	0	0
-88CT	3	2	0	0	0

the sickle cell β -thalassemia manifest variable clinical, hematological, and prognostic profiles. High level of fetal hemoglobin in patients reduces the severity of clinical symptoms in some but not in others. Coexistence of β -thalassemia mutation with sickle cell showed severe clinical manifestations only in those areas or communities where abnormal hemoglobin and β -thalassemia are highly prevalent (27). In India, the incidence of sickle gene varies from 0% to 40% and the relatively high frequency of β -thalassemia in same population groups often leads to the clinically important condition (28). The clinical and hematological features in sickle cell β -thalassemia are quite variable. Hemoglobin, MCV, MCH, HCT, and MCHC levels were low in our studied sickle cell β -thalassemia patients while these parameters were raised with the presence of α -globin gene deletions. Patients with α -triplication showed severe clinical phenotype as well as lowest hemoglobin levels and red cell indices. Mean value of hemoglobin ($P < 0.001$), reticulocytes ($P < 0.001$), and red cell indices were statistically significant in the compared groups. Coexistence of the α -thalassemia ($-\alpha$ 3.7/ α α) modified the expression of homozygosity for IVS1-6 mutation in such a way that there were higher hemoglobin and lower HbF levels as compared to those without α -thalassemia (29). A similar effect of the homozygosity of α -thalassemia on HbF value was previously observed in patients with sickle cell anemia (30). The predominant β -thalassemia mutation in this study was IVS1–5 (44%) and co-inherited α -deletion was 37.34%. Clinical features and hematological presentation of sickle β -thalassemia patient showed significant differences due to influence of α -gene numbers. Patient with α -triplication (Anti α -3.7kb) showed severe phenotype. Grading of anemia in β -thalassemia depends on the degree of globin chain imbalance. Free α -globin chain interacts with red cell precursors leading to ineffective erythropoiesis (31, 32). Patients with co-inherited α -globin gene deletions had less redundant α -globin chains and tend to present less severe phenotype. α -Chain deletions cause reduced intracellular concentration of sickled RBC, which leads to reduced rigidity, longer lifespan, raised HCT, and blood viscosity. These alterations have clinical beneficial effect on the

TABLE 2. α -Gene Numbers and Hematological Parameters of Patients

Hematological parameters	Mean $\pm$ SD			P-value
	S β thal. without α -del. (N = 43)	S β thal with α -del. (N = 28)	S β thal with α -trip. (N = 4)	
WBC (Ths/ μ l)	7.66 $\pm$ 3.61	8.13 $\pm$ 3.02	9.93 $\pm$ 2.1	0.57
RBC (millions/ μ l)	3.58 $\pm$ 0.86	4.72 $\pm$ 0.82	2.81 $\pm$ 0.5	<0.001
HGB (g/dl)	7.53 $\pm$ 1.46	9.39 $\pm$ 1.25	5.7 $\pm$ 3.24	<0.001
HCT (%)	27.42 $\pm$ 3.58	29.0 $\pm$ 3.06	28.12 $\pm$ 3.22	0.058
MCV (fl)	60.32 $\pm$ 2.91	65.49 $\pm$ 4.93	57.7 $\pm$ 1.39	<0.001
MCH (pg)	22.06 $\pm$ 1.66	25.78 $\pm$ 5.9	20.15 $\pm$ 2.06	<0.001
MCHC (g/dl)	27.88 $\pm$ 3.14	29.38 $\pm$ 3.08	24.42 $\pm$ 5.18	0.051
PLT (Ths/ μ l)	127.02 $\pm$ 58.56	140.96 $\pm$ 48.36	166.5 $\pm$ 95.61	0.298

TABLE 3. Frequency of Clinical Parameters in Presence of α -Gene Numbers

Clinical Parameters	Frequency (%)		
	S β thal without α -del. (N = 43)	S β thal with α -del. (N = 28)	S β thal with α -trip. (N = 4)
Anemia	55.81	46.42	100
Pallor	41.86	32.14	75
Weakness	39.53	21.42	25
Painful crises	37.7	25	75
Splenomegaly	34.88	17.85	50
Gallbladder stone	30.23	21.42	50
Fever	27.9	25	25
Chest pain	23.25	25	50
Hepatomegaly	23.25	25	50
Jaundice	20	14.28	50
Septicemia	9.3	3.57	25
Hyperpigmentation	9.3	7.14	25
Avascular necrosis (AVN)	6.97	7.14	None
Retinopathy	4.65	3.57	None
Deep vein thrombosis (DVT)	2.35	3.57	None

patients (33–35). Genetic variation of the globin gene mutations often caused by genetic modifiers and α -globin gene number can influence disease phenotype. In thalassemia major or intermedia, reduction in the number of α -globin genes can ameliorate the disease phenotype (36). Similar finding were reported in the patients of hemoglobin E β -thalassemia (37–40). The finding of this study is similar to our previous report on co-inheritance of α -chain deletions and modulating effect on the phenotype of sickle homozygous patients (41). Impaired production of α -globin chains leads to a relative excess of gamma globin chains, which is converted in β -globin chains. The excess β -globin chains are capable of forming soluble tetramers, which are unstable and precipitate within the cell, leading to a variety of clinical manifestations. β -Thalassemia conditions occur because of impaired production of β -globin chains, which leads to excess α -globin chains that cannot form soluble tetramers and thus precipitate. The excess α -globin

chains in β -thalassemia begin aggregating and accumulating in erythroid precursors. The clinical manifestations are generally less severe in α - compared to β -thalassemia because of aggregates of α -globin, except homozygous α -thalassemia (42). The defects in α -globin chain synthesis in α -thalassemia ameliorate β -thalassemia severity by rebalancing the α - to β -globin chain ratio. Molecular basis of clinical severity in patients with α -thalassemia needs a broad spectrum study in genetic defects of α -globin chain synthesis and phenotypes of patients (43). The observation of present study reveals that the presence of α -deletions influence the hematological and clinical features and produce mild phenotype while the triplification of α -gene worsen the phenotype in Indian sickle cell β -thalassemia patients.

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