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Short Communication

African Sickle Beta Zero-Thalassemia Patients Vs Sickle Cell Anemia Patients: Similar Clinical Features but Less Severe Hemolysis

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Abstract

Introduction: S-β zero thalassemia (Sβ0) represents less than 5% of the sickle cell disease (SCD) phenotypes. Although the Sβ0 phenotype is believed to be as severe as the homozygous (SS) sickle cell anemia, the two phenotypes have rarely been compared.

Objectives: To compare the clinical and biological features of Sβ0 and SS patients.

Methods: We conducted a nested case-control study within the CADRE cohort, including 3747 SCD patients in five sub-Saharan African countries. The SCD phenotype was established using hemoglobin electrophoresis results (i.e. absence of HbA, HbA2>3.5%) in combination with low mean glomerular volume (<78 fL). We studied the characteristics of 152 Sβ0 patients and 1353 SS patients matched by age and country and compared the two groups using a multivariate conditional regression analysis.

Results: There was no significant difference between the two groups in terms of clinical complications but Sβ0 patients displayed less frequent micro-albuminuria (24% vs 41%, p=0.02), a lower hemolysis level as assessed by the hemoglobin (8.8 vs 8.2 g/dL, p<0.0001), LDH (551 vs 732 IU/L, p=0.021) and bilirubin levels (23 vs 33 µmol/L, p=0.0001), and a higher percentage of fetal hemoglobin (14.5 vs 8.8%, p=0.002).

Conclusion: Overall, our study conducted in West Africa confirms the similarity between Sβ0 and SS phenotypes regarding most clinical features except for the hemolysis level and the glomerular involvement that are less severe in Sβ0 than in SS phenotype.

ABBREVIATIONS

SCD: Sickle Cell Disease; SS: Homozygous Genotype of Sickle Cell Disease; Hb: Hemoglobin; HbS/A0/A2/F: Hemoglobin S/A0/A2/F; Sβ0: S/β Zero-Thalassemia; PAH: Pulmonary Arterial Hypertension; eGFR: estimated Glomerular Filtration Rate; LDH: Lactate Dehydrogenase; USA: United States of America

INTRODUCTION

Sickle cell disease (SCD) results from a substitution of a glutamic acid by a valine at position 6 in the β-globin gene. When the mutation is homozygous (SS genotype), the hemoglobin (Hb) tetramer is constituted of 2 α chains and 2 S-β chains (HbS). The resulting SS phenotype represents 70% of the sickle cell

phenotypes [1]. In the S/β zero-thalassemia (Sβ0) genotype, one β-globin gene carries the S-β chain mutation whereas the other carries a null mutation conferring β-thalassemia trait, called β0 because given the inability to synthesize any β-globin chain. The Sβ0 phenotype accounts for less than 5% of SCD patients and is considered as severe as the SS phenotype [2]. However, the two phenotypes have rarely been compared: in most studies SS and Sβ0 phenotypes are pooled together. Although both genotypes result in a high amount of HbS and the absence of HbA0, biological differences may exist, possibly leading to different clinical outcomes. The aim of our study was to compare the clinical and biological features of patients with Sβ0 and SS phenotype in West Africa.

MATERIALS AND METHODS

We performed a case-control transversal study nested in the CADRE cohort, a large multinational cohort of SCD patients, currently conducted in five countries in sub-Saharan Africa [3]. SCD patients were recruited through outpatient clinics in two public and one private hospitals of Yaoundé, one public hospital in Douala (Cameroon), one university hospital in Abidjan (Ivory Coast), two university hospitals in Dakar (Senegal), the Centre International de Recherche Médicale de Franceville (Gabon), and the Centre de Recherche et de Lutte contre la Drépanocytose in Bamako (Mali). The study was announced through poster, radio and newspaper advertising to recruit patients that would not spontaneously seek for medical care at the hospital. SCD patients' associations were contacted and asked to encourage their members to participate in the study. Patients did not receive any financial incentive but were offered reimbursement of transportation cost and free medical examination, blood and urine biological tests and echocardiography. The list of CADRE investigators is provided in the Acknowledgment Section. Patients were enrolled from February 2011 to December 2013. The clinical visits were performed at a steady state (no infection or vaso-occlusive crisis for the last 15 days and no blood transfusion for the last three months). SCD diagnosis was confirmed by alkaline hemoglobin electrophoresis and/or high-performance liquid chromatography. The SS phenotype was defined by the presence of HbS without HbA0 and the Sβ0 phenotype by the presence of HbS without HbA0, a high HbA2 rate (> 3.5%) and a low mean corpuscular volume (<78 fL) without iron deficiency. For each patient, we recorded the medical history, clinical features and the urine and blood tests results. Echocardiography was performed: left ventricular ejection fraction was quantitated, and pulmonary arterial hypertension (PAH) was defined by tricuspid regurgitation jet velocity of more than 2.5 m/s as per current guidelines of the American Society of Echocardiography [4]. The CADRE protocol was approved by the national ethics committee in each participating country (N043/MSLS/CNER-dkn, N18 MS-SG-CNESS/2011, N12/40 MSAS/DS/CNERS, N016-CNE-SE-2011, and N023-CNER-GB_2011).

We matched the Sβ0 patients with all available SS patients of the same age (+/- 3 years until the age of 20, +/- 10 years for older adults) and country. We compared the characteristics of the SS and Sβ0 phenotypes using conditional logistic regression, with matching on age and country. The studied variables included: clinical findings, vaso-occlusive crises frequency; history of infectious diseases and vascular complications (stroke, leg ulcer, retinopathy, priapism, osteonecrosis and PAH); renal disease defined by a urine albumin on creatinine ratio> 30mg/g or estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m2; cell blood count; lactate dehydrogenase (LDH) and bilirubin levels, and left ventricular ejection fraction.

RESULTS

Among the 3747 SCD patients included in the cohort, 152 patients (4.1%) had an Sβ0 phenotype: 106 in Côte d'Ivoire, 39 in Mali, 5 in Senegal and 2 in Cameroon. After matching for age and country, 1353 SS patients were retained for further comparisons.

The demographical characteristics of the patients are described in the Table (1). There was no difference in terms of sex ratio, height, and weight and body mass index between the two phenotypes. Icterus was more frequent in SS patients (52% vs 36% in Sβ0, p=0.0002). Acute events history (vaso-occlusive crises, acute chest syndrome and infectious diseases) was similar in the two groups. There was a trend to fewer vascular complications (leg ulcer, priapism, stroke and osteonecrosis) in Sβ0 patients compared to SS patients (Table 2). Sβ0 patients displayed lower left ventricular ejection fraction by echocardiography (64% vs 67%, p=0.03) (Table 3).

Several biological differences were noted between the two phenotypes (Table 3). Glomerular filtration rate was lower in Sβ0 patients but none of them had renal failure (eGFR<60 ml/min/1.73m2) and there was a trend to less glomerular hyper filtration (eGFR>150 ml/min/1.73m2) in Sβ0 patients. Micro-albuminuria was significantly less frequent in Sβ0 patients than in SS patients (24% vs 41%, p=0.03). The mean hemoglobin level was higher in Sβ0 patients (8.8 vs 8.2 g/dL, p<0.0001), whereas most other cell blood counts (neutrophils, monocytes, reticulocytes and platelets) were lower in Sβ0 than in SS patients (Table 1). Bilirubin mean and LDH median levels were lower in Sβ0 patients (respectively 23 vs 33 µmol/L, p=0.0001 and 551 vs 732 IU/L, p=0.021). In addition to the expected higher percentage of HbA2, the median percentage of fetal hemoglobin (HbF) was also significantly higher in Sβ0 patients (14.5 vs 8.8%, p=0.002).

DISCUSSION

Our series is the largest to date describing patients with the Sβ0 phenotype as a specific population. As compared to the

Table 1: Demographical characteristics of SCD patients with $S\beta^0$ or SS phenotype.

Hemoglobin phenotype	Sβ0	SS
	N=152	N=1353
Country		
Cameroon, n (%)	2 (1.3)	335 (24.8)
Côte d'Ivoire, n (%)	106 (69.7)	229 (16.9)
Mali, n (%)	39 (25.7)	453 (33.5)
Senegal, n (%)	5 (3.3)	336 (24.8)
Male sex, n (%)	66 (43.4)	619 (45.8)
Age, years, (mean, SD)	15.4 (9.4)	16.3 (8.6)

Abbreviations: n: number, SD: Standard Deviation

Table 2: Clinical characteristics of SCD patients with Sβ⁰ or SS phenotype.

Hemoglobin phenotype	Sβ⁰ N=152	SS N= 1353	OR [$CI_{95\%}$]	P value
Clinical findings				
Body mass index, kg/m² (mean, SD)	16.2 (3.6)	16.6 (3.7)	1.12 [0.65; 1.91]	0.685
Icterus, n (%)	53 (36)	688 (52)	0.46 [0.31; 0.69]	0.002
Heart rate, bpm (mean, SD)	80 (19)	77 (12)	1.33 [0.75; 2.34]	0.333
Systolic BP, mmHg (mean, SD)	105 (13)	107 (12)	1.10 [0.87; 1.40]	0.439
Diastolic BP, mmHg (mean, SD)	61 (8)	60 (8)	1.12 [0.91; 1.38]	0.278
Medical history				
Age of clinical onset > 3 years, n (%)	94 (63.1)	723 (55.2)	1.51 [1.01; 2.26]	0.045
More than1 VOC last year, n (%)	53 (36.3)	610 (46.0)	1.28 [0.80; 2.02]	0.496
Acute chest syndrome, lifetime, n (%)	21 (15.1)	213 (16.3)	0.79 [0.45; 1.37]	0.402
Stroke, lifetime, n (%)	1 (0.7)	22 (1.6)	0.55 [0.05; 6.27]	0.631
Leg ulcer, lifetime, n (%)	7 (4.6)	95 (7.0)	1.42 [0.59; 3.43]	0.437
Priapism, lifetime, n (%)*	4 (6.1)	98 (15.8)	0.47 [0.15; 1.45]	0.189
Osteonecrosis, lifetime, n (%)	8 (5.3)	121 (8.9)	0.93 [0.41; 2.11]	0.866
Osteitis, lifetime, n (%)	2 (1.3)	82 (6.1)	0.33 [0.08; 1.44]	0.141
Meningitis, lifetime, n (%)	4 (2.6)	19 (1.4)	1.36 [0.41; 4.54]	0.615
Septicemia, lifetime, n (%)	1 (0.7)	17 (1.3)	0.39 [0.05; 3.31]	0.389

Odds ratio (OR) and 95% confidence interval ($CI_{95\%}$) are calculated using logistic regression with matching on age and country; OR for quantitative variables have been standardized. Quantitative parameters are given in mean (standard deviation, SD) in case on normal distribution, otherwise in median (interquartile range, IQR: 1st quartile-3rd quartile).

Abbreviations: BP: Blood Pressure; bpm: Beat per Min; SD: Standard Deviation; VOC: Vaso-Occlusive Crisis.

*percentages calculated in males only

Table 3: Laboratory findings of SCD patients with Sβ⁰ or SS phenotype.

Hemoglobin phenotype	Sβ⁰ N=152	SS N=1353	OR [$CI_{95\%}$]	P value
Biological findings				
Hemoglobin, g/dL (mean, SD)	8.8 (1.6)	8.2 (1.5)	1.54 [1.27; 1.87]	<.0001
Reticulocytes, G/L (median, IQR)	158 (41-222)	203 (153-259)	0.60 [0.37; 0.99]	0.045
Bilirubin, µmol/L (mean, SD)	23 (14.0)	33(19.6)	0.49 [0.35; 0.71]	0.0001
LDH, UI/L (median, IQR)	551 (411-747)	732 (529-1102)	0.56 [0.35; 0.92]	0.0206
Foetal hemoglobin, % (median, IQR)	14.5 (7-22)	8.8 (6-13)	3.27 [1.56; 6.88]	0.0018
Leukocytes, G/L (mean, SD)	10.1 (3.5)	12.3 (4.8)	0.52 [0.39; 0.68]	<.0001
Lymphocytes, G/L (median, IQR)	2.8 (2.2-3.7)	4.0 (3-5.4)	0.56 [0.35; 0.92]	0.042
Granulocytes G/L (mean,SD)	5.8 (2.8)	6.3 (3.2)	0.59 [0.47; 0.74]	<.0001
Platelets, G/L (mean, SD)	387 (148)	449 (173)	0.60 [0.48; 0.74]	<.0001
Urine albumin/ creatinine > 30mg/g, n (%)	8 (24)	380 (41)	0.38 [0.17; 0.87]	0.022
eGFR>150 mL/min/1.73m²(median, IQR)	90 (63)	538 (45)	0.61 [0.34; 1.10]	0.099
Echocardiographic findings				
LVEF, % (mean, SD)	64 (8)	67 (9)	0.50 [0.27; 0.92]	0.038
Pulmonary hypertension*, n (%)	1 (7.1)	58 (26.6)	0.27 [0.03; 2.18]	0.218

Odds ratio (OR) and 95% confidence interval ($CI_{95\%}$) are calculated using conditional logistic regression with matching on age and country; OR for quantitative variables have been standardized. Quantitative parameters are given in mean (standard deviation, SD) in case on normal distribution, otherwise in median (interquartile range, IQR: 1st quartile-3rd quartile).

Abbreviations: eGFR: Estimated Glomerular Filtration Rate (Schwartz formula for children<15 years old and CKD-epi for adults); LVEF: Left Ventricular Ejection Fraction; n: number; NA: Not Applicable; SD: Standard Deviation

*Pulmonary hypertension was defined by echocardiographic tricuspid regurgitation jet velocity> 2.5m/s.

patients from the same area and age with the SS phenotype, we observed very similar clinical features in Sβ0 patients. There was only a trend to fewer vascular complications (leg ulcer, priapism, stroke and osteonecrosis) for Sβ0 patients. Likewise, comparing SS and Sβ0 patients from the Cooperative Cell Study in the USA, Castro et al., found no difference in the incidence of acute chest syndrome [5], and Ohene-Frempong et al., observed a non-significant lower incidence of stroke in Sβ0 patients [6]. However, we observed a less frequent glomerular involvement in Sβ0 patients compared to SS patients. To our knowledge, glomerulopathy had never been investigated separately in Sβ0 patients before. We also noticed a lower left ventricular ejection fraction by echocardiography in Sβ0 patients compared to SS patients, still within normal range, that may be explained by a

lower cardiac output due to the higher hemoglobin level.

Contrasting with the similar clinical features, we observed a significantly lower level of chronic hemolysis in Sβ0 patients compared to SS patients, whether evaluated with clinical icterus, hemoglobin, bilirubin or LDH levels. Interestingly, the percentage of HbF was also significantly higher in Sβ0 patients, as observed in β-thalassemia [7]. The higher concentrations of HbF and HbA2 may contribute to less hemolysis in these patients. Indeed, it has been demonstrated that HbF and HbA2 lower the risk of HbS polymerization in red blood cells and it is now well established that the genetic persistence of HbF is a major modifying factor of SCD severity [8,9]. We can also hypothesize that the lower concentration of HbS in the Sβ0 red cells contributes to the lower risk of hemoglobin polymerization.

Patients with β-thalassemia major display ineffective erythropoiesis characterized by an arrest of maturation and increased apoptosis of erythroid precursors, due to the toxicity of unmatched α-globin chains aggregates [10]. Such phenomenon is poorly studied in SCD and has never been specifically studied in the Sβ0 genotype. The α-globin chains excess is certainly less marked than in β-thalassemia major, since α-globin chains can bind to S-globin chain. Moreover, although Sβ0 patients display lower reticulocyte counts than SS patients, they also display higher Hb levels, which argue against a more pronounced ineffective erythropoiesis in Sβ0 patients compared to SS patients. Nevertheless, it could be interesting to look for extra medullary erythropoiesis in Sβ0 patients.

We acknowledge on a series of limitations for our study. First, we defined the SCD groups by a phenotypic method (high level of HbA2 on hemoglobin electrophoreses and red cell microcytosis) because the sequencing of the β-globin gene is not available in most sub-Saharan African countries. However, our phenotypic criteria have already been used robustly in previous studies [5]. Second, the transversal design of our nested case-control study cannot exclude a mortality bias. Finally, although our study is the largest to date comparing SS and Sβ0 patients, the sample size remains modest, due to the rarity of the Sβ0 phenotype (4.1% in our cohort, mostly from Ivory Coast). Therefore, it is unknown whether the absence of significant difference in clinical complications between the two phenotypes is due to a lack of statistical power or if there is no true clinical difference. A true difference may however be advocated for, since Sβ0 patients have significantly lower hemolysis level and the hyper hemolytic profile has been linked to the prevalence of several vascular complications in SCD, especially PAH, leg ulcer and priapism [11]. Lower albuminuria rates in Sβ0 patients in our study support this hypothesis. Indeed, glomerular involvement has been associated to the hemolysis level in a previous study of the CADRE cohort and in other studies [3,12-15]. Although most studied patients were recruited in West Africa, because the Sβ0 phenotype is much more frequent in this region, the results were consistent in the four studied countries, including Cameroon which is located in central Africa. Therefore we have no reason to believe that our results cannot be extrapolated to the rest of Africa.

CONCLUSION

In conclusion, our study supports the equivalence of Sβ0 and SS phenotypes for most clinical features in West Africa. However, the glomerular involvement is less severe and the hemolysis level is lower in Sβ0 than in SS phenotype, which may influence the incidence of chronic SCD complications. This later hypothesis will be assessed during the follow-up of the CADRE cohort.

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