Kidney dysfunction and beta S-haplotypes in patients with sickle cell disease

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Objective: To investigate the association between kidney dysfunction and haplotypes in sickle cell disease.

Methods: A cohort of 84 sickle cell disease patients, treated in a public health service in Fortaleza, Brazil, was studied. Hemoglobin S haplotypes were obtained from 57 patients as they had recently received blood transfusions with 18 of them agreeing to undertake urinary concentrating ability and acidification tests. The glomerular filtration rate was estimated using the Modification of Diet in Renal Disease Study equation. Urinary concentration was evaluated utilizing the urinary and serum osmolality ratio (U/P_{osm}) after 12 hours of water deprivation. Urinary acidification was evaluated by measuring the urinary pH before and after the administration of oral $CaCl_2$. The analysis of the haplotypes of the beta S gene cluster was carried out by polymerase chain reaction-restriction fragment length polymorphism. The analysis of variance (ANOVA) test was used for multiple comparisons of means and the Newman-Keuls test was used to identify which groups were significantly different.

Results: The mean age of the patients was 33 ± 13 years with 64.2% being females. The glomerular filtration rate was normal in 25 cases (30%) and a rate > 120 mL/min was seen in 52 cases (62%). Urinary concentration deficit was found in all patients who underwent the test and urinary acidification in 22%. There was no significant difference when comparing patients with the Bantu/Bantu and Benin/Benin haplotypes. On comparing patients with the Central African Republic-haplotype however, a higher number had glomerular filtration rates between 60 and 120 mL/min.

Conclusion: There was no significant difference among sickle cell disease patients regarding the haplotypes and kidney dysfunction.

Keywords: Anemia, sickle cell; Haplotypes; Beta-globins; Hemoglobinopathies; Kidney function tests; Kidney/physiopathology

Introduction

The severity of the clinical manifestations in sickle cell disease (SCD) has been associated with the presence of specific hemoglobin S (Hb S) haplotypes[1,2]. The Senegal haplotype is associated with higher levels of fetal hemoglobin (Hb F) and milder symptoms, while the Benin is associated with moderate Hb F levels, and the Bantu or Central African Republic haplotype shows the lowest Hb F levels and more severe disease[3,4]. There are few studies investigating the possible influence of Hb S haplotypes on kidney dysfunction in SCD. Guasch et al. (1999), in a study with 76 adults with SCD (Hb SS) in the USA, reported that the coinheritance of microdeletions in one or two of the four alpha-globin genes (alpha-thalassemia) was associated with a lower prevalence of macroalbuminuria (13%) compared to patients with intact alpha-globin genes (40%). The authors found no association between albuminuria and beta-globin gene haplotypes [Central African Republic (CAR) versus non-CAR haplotypes][5]. The aim of this study was to investigate the association between kidney dysfunction and specific Hb S haplotypes in a cohort of patients from Brazil.

Methods

A cohort of 84 patients with clinical and laboratory diagnoses of SCD was studied. All patients were being treated in a public health service in Fortaleza, Northeast Region of Brazil from December 2010 to November 2011. The patients were selected in the outpatient clinic of the Hematology Service. The protocol of this study was reviewed and approved by the Research Ethics committee of the Hospital Universitário Walter Cantídio, Universidade Federal do Ceará, Fortaleza, Brazil.

Hb S haplotypes were obtained from 57 patients because the remaining experienced problems with the test or had recently received blood transfusions. Of the 57, 18 agreed to undertake urinary concentration and acidification tests. Those who agreed to participate in the study by giving their written informed consent were included unless they had any exclusion criteria such as being under 18 or older than 65 years old, had taken nephrotoxic drugs within the previous 30 days, were hypertensive (systolic blood pressure $\geq$ 140 mmHg or diastolic blood pressure $\geq$ 90 mmHg), and had diabetes mellitus, urinary tract infections, systemic lupus erythematosus or other collagenosis.

Laboratory tests were evaluated from the last medical visit. Glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease Study (MDRD) equation[6]. This equation was used to estimate the GFR because creatinine alone is not a good marker of renal function[6]. The determination of hematological parameters was carried out using an automated blood cell counter (Sysmex KX-21N, Roche). DNA was isolated from peripheral blood leukocytes following the Sambrook protocol[7].

The presence of Hb S was confirmed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), according to the method described by Saiki[8]. The analysis of the haplotypes of the beta S gene cluster was by PCR-RFLP, with the analysis of six polymorphic restriction sites (XmnI 5'γG, Hind III γG, Hind III γA, Hinc II yb, Hinc II 3'yband Hinf I 5'ß) according to the method of Sutton[9].

Besides GFR, renal tubular function was evaluated through urinary concentration and acidification tests in order to better evaluate renal function. Urinary concentrating ability was evaluated using the ratio of urinary to serum osmolality (U/P$_{osm}$) after 12 hours of water deprivation as previously described[10]. Urinary acidification was evaluated by measuring urinary pH before and after the administration of oral $CaCl_2$ (2 mEq/kg - T$_0$ and T$_4$)[11]. Acidification defects were determined by the inability to decrease U$_{pH}$ to less than 5.5 after the administration of the acid load.

Statistical analysis

The Graph Pad Prism (version 5.0) computer program was employed for statistical analysis. The Kolmogorov-Smirnov test was used to evaluate the normal distribution of continuous variables, Analysis of variance (ANOVA) was used for multiple comparisons of means and the Newman-Keuls test was utilized to identify which groups were significantly different. The Fisher exact test was used to compare proportions between the different haplotypes and clinical complications. The level of significance in the analysis was set for p-values < 0.05.

Results

A total of 84 patients were studied with a mean age of 33 ± 13 years (range: 19-67 years); 54 (64.2%) were female. The haplotypes found were: Bantu/Bantu (n = 26), Bantu/Benin (n = 16), Bantu/atypical (n = 6), Benin/Benin (n = 6) and Benin/atypical (n = 3).

The mean serum urea and creatinine were 20 ± 17 mg/dL and 0.7 ± 0.6 mg/dL, respectively. GFR was normal in 25 cases (30%). A GFR < 60 mL/min was found in seven cases (8%) and > 120 mL/min in 52 cases (62%). Urinary concentration deficit was found in 18 patients (100%) who underwent the test after water deprivation, and urinary acidification defect was seen in four cases (22%).

A further analysis was carried out of patients with the Bantu/Bantu (n = 26) and Benin/Benin (n = 6) haplotypes and comparing haplotypes grouped as Central African Republic (CAR - n = 48) and non-CAR (n = 9). There was no significant difference when comparing patients with Bantu/Bantu and Benin/Benin haplotypes (Table 1). When comparing CAR (n = 48) versus non-CAR haplotypes, a higher frequency of GFR (between 60 and 120 mL/min) was noted among CAR patients (Table 2).

Table 1 - Renal function of sickle cell disease patients according to the haplotype (Bantu/Bantu vs. Benin/Benin)

	Bantu/Bantu n (%)	Benin/Benin n (%)	p-value
Glomerular filtration rate			
< 60 mL/min	3 (11.5)	1 (16.6)	1.0
60-120 mL/min	7 (26.9)	-	0.29
> 120 mL/min	16 (61.5)	5 (83.3)	0.63
Urinary concentration deficit	5 (19.2)	-	0.55
Urinary acidification deficit	2 (7.6)	-	1.0

Table 2 - Renal function of sickle cell disease patients according to the haplotype (CAR vs. non-CAR)

	CAR n (%)	Non-CAR n (%)	p-value
Glomerular filtration rate			
< 60 mL/min	3 (6.2)	1 (11.1)	0.50
60-120 mL/min	16 (33.3)	-	0.04
> 120 mL/min	29 (60.4)	8 (88.8)	0.13
Urinary concentration deficit	13 (27)	-	0.1
Urinary acidification deficit	3 (6.2)	-	1.0

CAR: Central African Republic

Discussion

The present study analyzed renal function of SCD patients according to the haplotypes. Some abnormalities were found, including decreases in GFR (8% of cases), glomerular hyperfiltration (62%), urinary concentrating deficit (100%) and acidification deficit (22%). There was no significant difference on comparing patients with the Bantu/Bantu and Benin/Benin haplotypes.

A higher frequency of GFR between 60 and 120 mL/min was noted in the CAR haplotypes group of patients. Kidney dysfunction, including glomerular and tubular abnormalities, is one of the main chronic complications of SCD[12]. The chief renal alterations include urinary concentrating and acidification defects, and glomerular hyperfiltration, which can lead to glomerulosclerosis[12,13].

The exact pathophysiology of sickle cell nephropathy is still to be elucidated but it is known that the polymerization of erythrocytes in the renal medulla, a region that is apt for this phenomenon due to its low local oxygen pressure, low pH, and high osmolality, is implicated in kidney injury related to SCD[14]. The association between some haplotypes and clinical manifestations has been reported in previous studies. A recent study conducted with children in Rio de Janeiro, Brazil, found a higher incidence of cerebrovascular disease among children with the Bantu/atypical beta S-globin gene haplotype[15].

Oxidative stress and severe clinical manifestations have also been associated with beta S-globin gene haplotypes. In a study of 95 SCD children from Panama, high plasma lipid peroxidation levels and low superoxide dismutase plus glutathione reductase activities were associated with increased severity of clinical manifestations, corresponding mainly to patients with the Bantu

and Benin haplotypes[16]. Oxidative stress is an important feature of SCD and plays a significant role in the pathophysiology of hemolysis, vaso-occlusion and organ damage in SCD. Several mechanisms contribute to the high oxidative burden in sickle cell patients, including the excessive levels of cell-free hemoglobin with its catalytic action on oxidative reactions, characteristic recurrent ischemia-reperfusion events, a chronic pro-inflammatory state, and higher autoxidation of Hb S[17]. Reactive oxygen species (ROS) and the (end-)products of their oxidative reactions are potential markers of disease severity and could be targets for antioxidant therapies[17].

In a recent study performed in our region, the levels of Hb F were found to be lower among patients with the Bantu/Bantu haplotype (6.14 ± 3.46 mg/dL), in comparison with the levels of the Bantu/atypical (10.88 ± 2.78 mg/dL) and Benin/Benin haplotypes (8.56 ± 1.93 mg/dL)[2]. Hb F is known to protect against most pathological consequences in SCD due to its exclusion from the sickle hemoglobin polymer[18] and so lower levels of Hb F found in patients with the Bantu/Bantu haplotype may be responsible for the severity seen in these individuals.

The association between beta S-globin gene haplotypes and kidney disease has rarely been described in the literature. Guasch et al., in one of the few studies published on this issue, found macroalbuminuria in 22 of 76 (29%) SCD patients. The coinheritance of microdeletions in one or two of the four α-globin genes (α-thalassemia) was associated with a lower prevalence of macroalbuminuria (13%) versus patients with intact alpha-globin genes (40%; p-value = 0.01). They found no association between albuminuria and beta-globin gene haplotypes (CAR versus non-CAR haplotypes)[5]. In the present study we also found no significant association between these haplotypes and renal abnormalities, maybe due to the high prevalence of the Bantu and Benin haplotypes, which are both associated with disease severity. The comparison of these patients with different haplotypes, such as the Senegal and Arab-Indian haplotypes, could show some differences but these haplotypes are rare in Brazil[19].

Conclusion

Renal abnormalities are common in patients with SCD. The most common are renal concentrating deficit and glomerular hyperfiltration. Further studies are required to better establish the relationship between beta-globin gene haplotypes and renal manifestations in SCD.

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