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Renal dysfunction in patients with sickle cell anemia or sickle cell trait

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Abstract

Patients with sickle cell anemia (Hb SS) or sickle cell trait (Hb AS) may present several types of renal dysfunction; however, comparison of the prevalence of these abnormalities between these two groups and correlation with the duration of disease in a large number of patients have not been thoroughly investigated. In a cross-sectional study using immunoenzymometric assays to measure tubular proteinuria, microalbuminuria, measurement of creatinine clearance, urinary osmolality and analysis of urine sediment, we evaluated glomerular and tubular renal function in 106 adults and children with Hb SS (N = 66) or Hb AS (N = 40) with no renal failure (glomerular filtration rate (GFR) >85 ml/min). The percentage of individuals with microalbuminuria was higher among Hb SS than among Hb AS patients (30 *vs* 8%, P<0.0001). The prevalence of microhematuria was similar in both groups (26 *vs* 30%, respectively). Increased urinary levels of retinol-binding protein or $ß_2$-microglobulin were detected in only 3 Hb SS and 2 Hb AS patients. Urinary osmolality was reduced in patients with Hb SS or with Hb AS; however, it was particularly evident in Hb SS patients older than 15 years (median = 393 mOsm/kg, range = 366-469) compared with Hb AS patients (median = 541 mOsm/kg, range = 406-722*)*. Thus, in addition to the frequently reported early reduction of urinary osmolality and increased GFR, nondysmorphic hematuria was found in 26 and 30% of patients with Hb SS or Hb AS, respectively. Microalbuminuria is an important marker of glomerular injury in patients with Hb SS and may also be demonstrated in some Hb AS individuals. Significant proximal tubular dysfunction is not a common feature in Hb SS and Hb AS population at this stage of the disease (i.e., GFR >85 ml/min).

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Key words
• Microalbuminuria
• Renal failure
• Renal function
• Sickle cell disease

Introduction

The kidney of the homozygous sickle cell anemia (Hb SS) patient is affected by the hemodynamic changes of chronic anemia and by the consequences of vaso-occlusion, especially in the renal medulla (1,2). The disruption of distal nephron and medullary function leads to a reduction of renal concentrating capacity, urinary acidification, and to impaired potassium metabolism which are often observed in these patients (3,4). In contrast, glomerular function is usually well maintained. In children and young adults the glomerular filtration rate (GFR) exceeds the normal value; however, there is a gradual decrease in GRF which may result in chronic renal failure associated or not with glomeru-

lar changes. The contribution of the deteriorating glomerular function to morbidity and mortality in older patients has not been widely recognized (5,6). Proteinuria has been described alone or in association with renal vein thrombosis, hematuria, leg ulcers, acute or chronic glomerulonephritis, and the other components of the nephrotic syndrome (6). The prevalence rate of proteinuria in patients with Hb SS has been reported to vary from 17 to 33% in studies in which proteinuria was determined by the dipstick method (7). While asymptomatic and relatively common, proteinuria seems to be associated with reduced creatinine clearance in Hb SS patients older than 40 years.

In the present study we determined the prevalence of early glomerular and proximal tubular dysfunction in individuals with either Hb SS or sickle cell trait (Hb AS). In addition, the possible correlation between renal dysfunction and duration of disease was investigated.

Subjects and Methods

A cross-sectional study was conducted on 106 individuals, 66 patients with documented Hb SS (median age: 22 years (2 to 49 years)) and 40 individuals with Hb AS (median age: 28 years (5 to 69 years)). All subjects were followed at the Hematology Service of Escola Paulista de Medicina and Faculdade de Ciências Médicas da Santa Casa de Misericórdia, São Paulo, SP, Brazil. The exclusion criteria were: documented acute or chronic infection, painful crisis, or hemodynamic instability. The presence of infection was assessed by medical history, clinical examination and, when indicated, laboratory tests such as complete blood count, urinalysis (urine cultures were usually done if there was leucocyturia >10,000 cells/mm^3), blood cultures, chest X ray, abdominal ultrasonography, etc. The diagnosis of Hb SS or Hb AS was based on clinical features, family history, and laboratory investigation. Laboratory techniques included hemoglobin electrophoresis on cellulose acetate, pH 8.9, and on agar gel, pH 6.2 (8), the solubility test (9), and the estimation of both Hb F (10) and Hb A_2 (8). The individuals with Hb AS were either parents or siblings of the patients with Hb SS included in the study.

Total protein in urine was measured by the biuret method (11) and the level of serum creatinine was determined by the Jaffé reaction (12). Creatinine clearance determinations were based on 24-h urine collection and were calculated as follows: (urine creatinine x 24-h urine volume)/plasma creatinine. Patients were carefully instructed on how to collect urine at home and bring it to the laboratory at the end of the procedure. All creatinine clearance results were adjusted for body surface area (1.73 m^2). The level of $ß_2$-microglobulin in serum or urine was determined using a commercially available kit (Enzygnost $ß_2$-microglobulin, Behring Institute, Mannheim, Germany, upper normal limit = 200 µg/l). A monoclonal antibody-based immunoenzymometric assay was used to determine urinary retinol-binding protein (RBP, upper normal limit = 400 µg/l) (13). Microalbuminuria was quantitated by ELISA (upper normal limit = 20 mg/l). Urinary osmolality was measured by the freezing point depression method using a 3W2 osmometer (Advanced Instruments Inc., Needham Heights, MA). $ß_2$-Microglobulin, RBP, microalbuminuria and urinary osmolality (after 12-16 h dehydration) were determined in urine samples. Previous studies from our institution have shown an excellent correlation between urinary RBP (or $ß_2$-microglobulin) expressed as µg/l and the respective values corrected for urinary creatinine, when renal function is preserved, as was the case for the present patients (13). We preferred to express the results of these parameters as µg/l. Hematuria was defined as the presence of 8 or more red blood cells per high power microscopic field in the urine sediment. If there was hematuria, an evaluation of eryth-

rocyte morphology was carried out using phase contrast microscopy.

Statistical analyses

Statistical analyses were performed using nonparametric methods. The Mann-Whitney test was used for the comparison of continuous variables, while the chi-square test was used for the comparison of categorical variables, with the level of significance set at 0.05 (two-tailed).

Results

Table 1 summarizes the laboratory results obtained for the two groups of individuals. Hb SS patients had a significantly lower level of serum creatinine and urinary osmolality than Hb AS individuals. In contrast, patients with Hb SS showed a significantly higher level of creatinine clearance, microalbuminuria, and serum $ß_2$-microglobulin than individuals with Hb AS.

The percentage of individuals with microalbuminuria was significantly higher in Hb SS than Hb AS patients (30 *vs* 8%, P<0.0001). However, the presence of hematuria was quite similar in both groups (26 *vs* 30%). In the group of patients with Hb AS we detected one patient showing gross hematuria and another having dysmorphic erythrocytes in the urine.

Seven patients with Hb SS and 2 patients with Hb AS showed a 24-h total proteinuria level of 0.2 to 1.0 g, whereas one Hb SS patient presented 24-h proteinuria of 1.65 g. None of these patients showed clinical evidence of nephrotic syndrome (edema). Increased values of urinary RBP and $ß_2$-microglobulin were detected in 1 and 3 patients with Hb SS, respectively. Two individuals with Hb AS had increased levels of $ß_2$-microglobulin, but none had elevated urinary RBP.

The results of the laboratory parameters

Table 1 - Renal function parameters observed in patients with sickle cell trait (Hb AS) and sickle cell anemia (Hb SS).

Values are reported as median (range). RBP = Retinol-binding protein; ns = not significant. *8000 cells/field.

Parameter	Hb AS	Hb SS	P Value
Number of patients	40	66	-
Median age (range) (years)	28 (5-69)	22 (2-49)	ns
Female/male ratio	32/8	40/26	-
Serum creatinine (mg/dl)	0.70 (0.4-1.1)	0.60 (0.3-1.3)	<0.01
Creatinine clearance (ml/min/1.73 m^2)	108 (86-212)	118 (87-229)	<0.01
Urinary osmolality (mOsm/kg)	567 (333-829)	399 (271-580)	<0.001
Microalbuminuria (mg/l)	6.8 (0.8-364.0)	10.5 (0.7-2022.0)	<0.05
Number of patients with higher than normal values (%)	3/39 (8)	19/64 (30)	<0.0001
Hematuria (cells/field, 400X)	3 (0-80)	3 (0-15)	ns
Number of patients with higher than normal values* (%)	12/40 (30)	17/66 (26)	ns
Urinary RBP (µg/l)	35 (5-261)	45 (7-462)	ns
Number of patients with higher than normal values (%)	0/34 (0)	1/49 (2)	ns
Urinary $ß_2$-microglobulin (µg/l)	55 (12-224)	72 (15-761)	ns
Number of patients with higher than normal values (%)	2/40 (5)	3/64 (5)	ns
Serum $ß_2$-microglobulin (mg/l)	1.39 (0.58-2.59)	1.92 (0.82-3.65)	<0.0001

according to age group for the two groups of individuals are shown in Table 2. In the Hb SS group, patients older than 15 years showed a significantly higher level of serum creatinine and microalbuminuria excretion compared to the group of Hb SS patients under 15 years of age. However, Hb SS patients over 15 years of age showed a significantly lower urinary osmolality than Hb SS patients under 15 years. All 19 (49%) Hb SS patients with microalbuminuria were over 15 years of age (Figure 1).

In Hb SS patients, the median (range) Hb F value was similar for subjects with or without microalbuminuria, i.e., 5.1% (1.8-8.4) *vs* 5.3% (0.3-20.3), respectively. In these subgroups (with or without microalbuminuria), median (range) creatinine clearance was also not significantly different, i.e., 133 ml/min (96-169) *vs* 118 ml/min/1.73 m^2 (87-192), respectively.

Urinary osmolality was reduced in both patients with Hb SS and with Hb AS, but this reduction was particularly evident in Hb SS patients over 15 years of age.

Discussion

In the present study we evaluated the renal function of a relatively large number of adults and children with either Hb SS or Hb AS. It has been reported that patients with Hb SS may present several types of renal dysfunction including hyposthenuria, hematuria, proteinuria, nephrotic syndrome, acidosis, urinary tract infection, renal failure, and changes in arterial blood pressure (6). The inability to concentrate urine appropriately has been the most consistent feature of sickle cell nephropathy which has been reported to occur in all sickle cell disorders, including Hb AS (6,14). Typically, adult Hb SS patients show a maximum urinary concentration of about 400 mOsm/kg (15). It has been suggested that this defect in urinary concentration can be restored to normal in very young children with Hb SS submitted to multiple transfusions of Hb AA erythrocytes (16). The present data confirm the results described in previous reports showing an early and progressive inability to concentrate urine in Hb SS which was also present to a lesser extent in Hb AS individuals. The generally accepted concept is that the gross destruction of the vasum rectum system impairs tubular function so that the high medullary interstitial solute concentration necessary for urinary concentration cannot be gen-

Table 2 - Laboratory data of patients with sickle cell trait (Hb AS) and sickle cell anemia (Hb SS) according to age group.

Values are reported as median (range). RBP = Retinol-binding protein; ns = not significant. P values correspond to the comparison between age groups; #P<0.05; ##P<0.01; *P<0.001; **P<0.0001 for the comparison with the corresponding Hb AS age group (Mann-Whitney test).

Parameter	Hb AS			Hb SS		
	≤15 years	>15 years	P Value	≤15 years	>15 years	P Value
Number of patients	13	27		27	39	
Serum creatinine (mg/dl)	0.60 (0.4-0.8)	0.80 (0.4-1.1)	<0.002	0.50# (0.3-0.8)	0.60# (0.4-1.3)	<0.001
Creatinine clearance (ml/min/1.73 m^2)	103 (97-122)	110 (86-212)	ns	114 (87-192)	124## (111-229)	ns
Urinary osmolality (mOsm/kg)	607 (333-829)	541 (406-772)	ns	419* (365-580)	393** (366-469)	<0.001
Microalbuminuria (mg/l)	4.0 (0.8-65.4)	6.8 (0.8-364.0)	ns	5.40 (0.7-12.7)	19.3** (1.2-2022)	<0.001
Hematuria (cells/field, 400X)	5 (0-80)	3 (0-10)	ns	2 (3-10)	4 (1-80)	ns
Urinary RBP (µg/l)	43 (5-261)	34 (6-124)	ns	37 (7-129)	64 (9-462)	<0.05
Urinary $ß_2$-microglobulin (µg/l)	77 (12-224)	52 (17-206)	ns	60 (15-167)	81 (15-761)	ns
Serum $ß_2$-microglobulin (mg/l)	1.41 (0.85-2.20)	1.35 (0.58-2.59)	ns	1.65 (0.83-2.22)	2.02* (0.82-3.65)	ns

erated or maintained (1).

Gross or microscopic hematuria has been detected, usually involving the left kidney, and at times severe enough to endanger the patient's life or be confused with renal malignancy (6). In this study, the percentage of patients with microscopic hematuria was relatively high in both Hb SS and Hb AS groups, being slightly more frequent in the latter (26 and 30%, respectively). Hematuria was basically of nonglomerular origin, since only in one patient did we find dysmorphic urinary erythrocytes. It has been assumed that hematuria is produced by increased sickling of the erythrocytes in the medulla of the kidney. This causes sludging of the blood in the inner medulla with resulting ischemia and extravasation. At least three factors known to cause sickling could be operative in the medulla of the kidney: increased acidity, hypertonicity and anoxia.

In children with Hb SS, renal function is characterized by glomerular hyperfiltration and hyperperfusion but, as early as the second decade of life, GFR often declines, despite the persistence of high renal blood flow rates (17). Chronic renal failure has been increasingly diagnosed in Hb SS patients and, in some countries, it is one of the most common causes of death among Hb SS patients over 40 years of age (2,18). In the present study, patients did not have significantly reduced GFR. Glomerular hyperfiltration (GFR >140 ml/min) was more commonly observed in Hb SS than in Hb AS individuals.

It has been reported that patients with Hb SS may present proximal tubule hyperfunction. In fact, increased proximal tubular reabsorption of $ß_2$-microglobulin was found in patients with Hb SS, resulting in elevated serum levels of the protein. There seems to be a close relationship between clearance of $ß_2$-microglobulin and phosphate clearance, both markers of proximal tubular function (1,3). In addition, other markers of tubular dysfunction may be detected in some patients such as an acidification defect (renal tubular acidosis), hyperuricemia and hyperkalemia (1,3). The great majority of our patients had urinary ß2-microglobulin and RBP concentrations within the normal range. Only 3 Hb SS and 2 Hb AS patients had slightly increased urinary excretion of these microproteins, suggesting that significant proximal renal injury is quite uncommon in Hb SS and Hb AS patients.

We were unable to detect any relationship between Hb F levels and microalbuminuria. As we have reported earlier, the Hb F levels in Brazilian patients with Hb SS is relatively low (median = 6.7%, range = 0.7-19.2) to protect against severe systemic complications of the disease (19). The higher incidence of the CAR/CAR haplotype in our Hb SS patients could explain the low level of Hb F observed (20).

We found a high prevalence of microalbuminuria in Hb SS patients (30%), mainly in those with a longer duration of disease (49% in patients older than 15 years). In some of these patients (N = 8) 24-h total proteinuria was also increased. In addition, microalbuminuria was detected in 3 Hb AS individuals. However, no significant correlation was detected between microalbuminuria and GFR. It is noteworthy that although patients in this cohort had preserved renal function (creatinine clearance), microalbuminuria was detected in many of them. Possible mechanisms of microalbuminuria are related to a reduction of ultrafiltration coefficient and loss of glomerular permselectivity. Microalbuminuria seems to be an

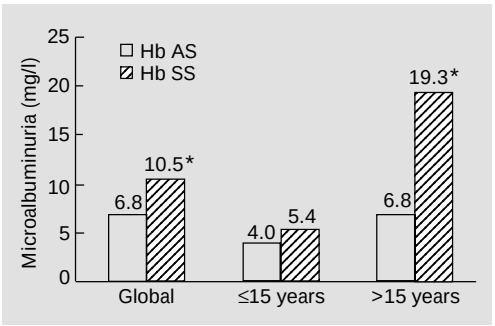

Figure 1 - Microalbuminuria levels in patients with sickle cell trait (Hb AS) and sickle cell anemia (Hb SS) according to age. *P<0.01 for the global comparison between Hb AS and Hb SS groups, and for the comparison between subgroups over 15 years (Hb AS *vs* Hb SS) (Mann-Whitney test).

early indicator of glomerular injury that could precede the development of glomerulosclerosis or other types of glomerulonephritis. In fact, recently, Guasch et al. (17) showed that when albuminuria is present, the ultrafiltration coefficient is already diminished even if GFR is preserved. Therefore, the detection of microalbuminuria seems to be an important marker of glomerular renal injury in patients with Hb SS.

In conclusion, in this extensive survey of patients with Hb SS without chronic renal failure we observed that microalbuminuria was frequently detected in Hb SS patients (half of those older than 15 years), and to a lesser extent in Hb AS individuals. Microhematuria was another common feature in both groups. Proximal tubular dysfunction was not a relevant finding.

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