Serum urate concentrations in homozygous sickle cell disease

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SUMMARY Serum and urinary urate concentrations were studied in 44 patients with homozygous sickle cell (SS) disease, and in 27 controls with normal haemoglobin. Hyperuricaemia (>0.39 mmol/l (6.5 mg/100 ml)) occurred in 41% of SS patients and inversely correlated with renal urate clearance but not with indices of bone marrow turnover. Higher serum urate concentrations occurred in patients with proteinuria, probably due to associated tubular damage. Higher serum urate concentrations and lower urate clearance occurred in males compared to females.

Hyperuricaemia is a common feature of homozygous sickle cell (SS) disease.^{1,2} Its clinical significance is not clear, as gout is considered to be rare in this disease.^{3,4} Factors contributing to hyperuricaemia in SS disease include the markedly increased red cell turnover,⁵ decreased renal excretion,^{2,6} and perhaps diminished uricolysis in the gut. This study was undertaken to determine the relative contribution of haematological factors and of renal excretion to the serum urate concentrations in a group of SS outpatients on an unrestricted diet.

Patients and methods

PATIENTS

Patients were drawn from routine attendances at the Sickle Cell Clinic of the University Hospital of the West Indies. There were 44 patients with SS disease (23 male, 21 female) with ages ranging from 9-61 yr (mean $\pm$ SD, 25.6 ± 10.7 yr). All were in the steady state—that is, without acute complications, at the time of examination but proteinuria of more than 0.1 g/24 h was present in 7 (3 male, 4 female). None was in renal failure (range of serum creatinine: 20 - 110 μ mol/l (0.22 - 1.24 mg/100 ml)). Criteria for the diagnosis of SS disease, which have been reviewed elsewhere,⁷ included haemoglobin electrophoresis on cellulose acetate and agar gel, quantification of HbA₂, and family studies where possible.

Controls were healthy West Indian medical and laboratory staff of predominantly African origin with normal haemoglobin electrophoresis (AA genotype). There were 27 controls (11 male, 16

female) with ages ranging from 13-49 yr (mean $\pm$ SD, 28.9 ± 9.4 yr). No patients or controls were taking any medication at the time of the study.

METHODS

A single mid-morning urine sample and concurrent serum sample taken from each patient and control were stored at -20°C and thawed out before analysis. No dietary restrictions were imposed.

Urate concentration in serum and urine were measured colorimetrically on the Technicon AA1 autoanalyser, paired samples having a variation of 3%. No consistent changes were observed between frozen samples and freshly analysed specimens, values differing by up to 0.02 mmol/l (0.33 mg/100 ml). The increased bilirubin concentration common in SS disease was shown not to interfere with the estimation of urate, at bilirubin concentrations up to 300 μ mol/l (17.5 mg/100 ml).

Creatinine in urine and serum was estimated by the alkaline picrate method on the Technicon SMA 6/60 autoanalyser by the reduction of phosphotungstic acid.

The urate clearance (C_u) corrected for glomerular filtration rate as measured by endogenous creatinine clearance (C_{cr}) was calculated from the equation:

$$C_u/C_{cr} = \frac{\text{urinary urate} \times \text{serum creatinine}}{\text{serum urate} \times \text{urinary creatinine}}$$

Urate excretion was measured by calculation of the urinary urate:creatinine ratio (U_u/U_{cr}),⁸ which was preferred to the alternative index (U_u/C_{cr})⁹ since it relates urate excretion to muscle mass, and hence body size, and is independent of glomerular function, which may vary widely in SS disease.¹⁰

Hyperuricaemia was defined as a serum concentration of more than 0.39 mmol/l (6.5 mg/100 ml) at which the serum becomes supersaturated with urate.¹¹ Sickle cell patients were divided in normouricaemic and hyperuricaemic groups according to this definition.

A blood sample taken at the same time as the urine sample was used for determination of total haemoglobin, reticulocyte count, percentage of fetal haemoglobin and total bilirubin.

STATISTICAL METHODS

Values of C_u/C_{cr} showed a skewed distribution which was corrected by log transformation. Other values were normally distributed and were analysed directly. Means were compared by Student's *t* test for unpaired data, correlations were evaluated by linear regression analysis, and contingency tables by the χ^2 test.

Results

SERUM URATE

Serum urate concentrations (Table 1) were higher in patients with SS disease than in normal controls ($p < 0.001$), and higher in SS patients with proteinuria than in those without ($p < 0.001$). Hyperuricaemia occurred in 18/44 (41%) patients and in no controls. Hyperuricaemia occurred in 6/7 (89%) of patients with proteinuria, in contrast with 12/37 (32%) of those without proteinuria. ($\chi^2 = 4.9$, $p < 0.05$).

Male patients had higher serum urate concentrations than females ($p < 0.001$) and a greater proportion of males were hyperuricaemic (45% compared to 18%). Four patients, all male, showed hyperuricaemia before the age of 20 yr. The youngest female SS patient with hyperuricaemia was 23 yr.

URATE CLEARANCE

There was no significant difference in C_u/C_{cr} (Table 2) between patients and controls. However when patients were divided in normouricaemic and hyperuricaemic groups, mean C_u/C_{cr} in the hyperuricaemic group (4.04) was significantly lower than in the normouricaemic group (7.32) ($p < 0.02$).

Serum urate concentrations correlated inversely with C_u/C_{cr} ($r = -0.49$, $p < 0.01$), in males ($r = -0.52$, $p < 0.02$) but not in females ($r = -0.37$, NS) (Figure).

Values of C_u/C_{cr} were consistently higher in females in all subgroups of SS disease ($p < 0.05$) and in normal controls ($p < 0.02$).

In SS patients with proteinuria mean C_u/C_{cr} was lower than in those without proteinuria but the difference did not reach statistical significance (3.93 vs 6.04, $p > 0.05$).

URATE EXCRETION

In SS patients, U_u/U_{cr} was greater than in controls ($p < 0.001$, Table 3). Raised ratios (> 0.5 , 2SD above the mean ratio in controls) occurred in 16/44 (36%) SS patients, all but one of these were normouricaemic. In hyperuricaemic patients U_u/U_{cr} was lower

Table 1 Serum urate concentrations in patients with SS disease and in controls (mmol/l)

Group	Males		Females		Males and females	
	n	Mean $\pm$ SD	n	Mean $\pm$ SD	n	Mean $\pm$ SD
All SS patients	23	0.44 $\pm$ 0.12	21	0.35 $\pm$ 0.1	44	0.40 $\pm$ 0.12
Without proteinuria	20	0.41 $\pm$ 0.07	17	0.32 $\pm$ 0.08	37	0.37 $\pm$ 0.08
With proteinuria	3	0.64 $\pm$ 0.20	4	0.47 $\pm$ 0.11	7	0.54 $\pm$ 0.17
AA controls	11	0.33 $\pm$ 0.04	16	0.28 $\pm$ 0.05	27	0.30 $\pm$ 0.05

Conversion: SI to traditional units:
Urate 1 mmol/l $\approx$ 16.8 mg/100 ml.

Table 2 Urate clearance (C_u/C_{cr}) in patients with SS disease and in controls

Group	Males			Females			Males and females		
	n	Mean $\pm$ SD	Antilog of mean	n	Mean $\pm$ SD	Antilog of mean	n	Mean $\pm$ SD	Antilog of mean
All SS patients	33	1.49 $\pm$ 0.68	4.42	21	2.00 $\pm$ 0.61	7.36	44	1.73 $\pm$ 0.69	5.64
SS without proteinuria	20	1.59 $\pm$ 0.65	4.89	17	2.05 $\pm$ 0.62	7.74	37	1.80 $\pm$ 0.67	6.04
Hyperuricaemic	9	1.26 $\pm$ 0.75	3.54	3	1.80 $\pm$ 1.03	6.03	12	1.40 $\pm$ 0.81	4.04
Normouricaemic	11	1.85 $\pm$ 0.43	6.36	14	2.10 $\pm$ 0.55	8.17	25	1.99 $\pm$ 0.50	7.32
SS with proteinuria	3	0.82 $\pm$ 0.54	2.27	4	1.78 $\pm$ 0.56	5.94	7	1.37 $\pm$ 0.72	3.93
AA controls	11	1.46 $\pm$ 0.57	4.32	16	2.00 $\pm$ 0.50	7.36	27	1.78 $\pm$ 0.59	5.93

All means and standard deviations presented as logarithmic values after multiplying $\times 100$.

Relation between serum urate concentrations and urate clearance (C_u/C_{cr}) in male and female patients.
● without proteinuria. ○ with proteinuria

than compared to normouricaemic ones ($p < 0.02$), but there was no difference between SS patients with proteinuria and without. There was no sex difference in U_u/U_{cr} and no correlation with serum urate.

HAEMATOLOGICAL INDICES

The haematological indices are summarised in Table 4. As expected all patients were anaemic (Hb range 5.7-10 g/dl), with raised reticulocyte counts (range 4-18%) and bilirubin concentrations (range

17-245 $\mu\text{mol/l}$ (1.0-14.3 mg/100 ml)). Haemoglobin F concentrations varied from 0.1 to 8.6%. These values did not differ in normouricaemic and hyperuricaemic groups, but the patients with proteinuria had a lower haemoglobin than those without proteinuria ($p < 0.05$). Haemoglobin was inversely correlated with U_u/U_{cr} ratios in normouricaemic male ($r = -0.82$, $p < 0.001$), but not in female patients. Serum urate did not correlate with reticulocyte counts, or HbF concentrations which are both related to rate of red cell turnover. There was no relation between serum urate and bilirubin concentrations.

Discussion

The occurrence of hyperuricaemia, defined as a serum urate concentration above 0.39 mmol/l (6.5 mg/100 ml), in 41% patients is in close agreement with another study,⁶ and confirms that hyperuricaemia is a common feature of SS disease.^{1,2} Theoretically hyperuricaemia may result from increased production of uric acid, decreased excretion, or a combination of both. Studies of red cell survival and endogenous carbon monoxide production during the steady state in SS disease have suggested that the

Table 3 Urate excretion (U_u/U_{cr}) in patients with SS disease and in controls

Group	Males		Females		Males and females	
	n	Mean $\pm$ SD	n	Mean $\pm$ SD	n	Mean $\pm$ SD
All SS patients	23	0.43 $\pm$ 0.21	21	0.43 $\pm$ 0.21	44	0.46 $\pm$ 0.21
SS without proteinuria	20	0.45 $\pm$ 0.22	17	0.52 $\pm$ 0.20	37	0.48 $\pm$ 0.21
Hyperuricaemic	9	0.31 $\pm$ 0.14	3	0.53 $\pm$ 0.32	12	0.36 $\pm$ 0.21
Normouricaemic	11	0.57 $\pm$ 0.20	14	0.51 $\pm$ 0.19	25	0.54 $\pm$ 0.19
SS with proteinuria	3	0.28 $\pm$ 0.09	4	0.45 $\pm$ 0.19	7	0.38 $\pm$ 0.17
AA controls	11	0.22 $\pm$ 0.14	16	0.29 $\pm$ 0.09	27	0.26 $\pm$ 0.12

Table 4 Haematological indices in patients with SS disease

Group	n	Age (mean)	Hb (g/dl)	Reticulocytes (%)	Hb F (%)	Total bilirubin ($\mu\text{mol/l}$)
All patients	44	26	7.8 $\pm$ 1.0	12.7 $\pm$ 7.6	3.6 $\pm$ 2.2	76.2 $\pm$ 49.7
Hyperuricaemic						
Male	9	24	8.4 $\pm$ 1.0	10.8 $\pm$ 3.2	4.2 $\pm$ 2.0	71.5 $\pm$ 29.1
Female	3	34	7.2 $\pm$ 0.5	14.0 $\pm$ 3.5	4.7 $\pm$ 2.3	60.4 $\pm$ 21.7
Male and female	12	27	8.1 $\pm$ 1.1	11.6 $\pm$ 3.5	4.4 $\pm$ 2.0	68.7 $\pm$ 27.0
Normouricaemic						
Male	11	23	7.7 $\pm$ 0.9	16.3 $\pm$ 13.6	2.5 $\pm$ 1.0	87.7 $\pm$ 66.9
Female	14	24	8.1 $\pm$ 1.0	11.1 $\pm$ 3.9	4.0 $\pm$ 3.1	76.8 $\pm$ 55.9
Male and female	25	24	7.9 $\pm$ 1.0	13.4 $\pm$ 9.6	3.4 $\pm$ 2.5	81.6 $\pm$ 59.9
With proteinuria						
Male	3	30	7.1 $\pm$ 1.7	14.3 $\pm$ 3.2	3.6 $\pm$ 2.2	94.1 $\pm$ 64.5
Female	4	31	6.9 $\pm$ 0.7	10.5 $\pm$ 4.2	2.5 $\pm$ 1.2	56.1 $\pm$ 15.9
Male and female	7	31	7.0 $\pm$ 1.1	12.1 $\pm$ 4.1	3.0 $\pm$ 1.7	72.3 $\pm$ 44.0

Conversion: SI to traditional units
Bilirubin 1 $\mu\text{mol/l} \approx 0.058$ mg/100 ml.

marrow is functioning at about six times the normal rate.⁵ Increased nucleic acid breakdown results in increased production of uric acid,¹² which can be estimated by the 24-h urate excretion on a purine free diet¹³ or by calculation of U_u/U_{cr} on spot urine samples.⁸

Since all patients with SS disease in the steady state have evidence of increased erythropoietic activity, increased synthesis of uric acid might be expected. Although direct estimates of red cell survival were not available in this group, the close relation between slope of the red cell survival curve and steady state reticulocyte count previously observed ($n = 42$, $r = +0.67$, $p < 0.001$; unpublished observations) suggest that the steady state reticulocyte count reflects the degree of erythropoietic activity. It is therefore surprising that there was no clear relation between serum urate and reticulocyte count, and that only 36% of patients had increased urinary excretion of urate. The latter discrepancy was also noted by Diamond *et al.*⁶ who suggested that increased intestinal uricolysis in SS disease could lead to underestimation of urate production. However, our observations that only one of the 16 patients with raised U_u/U_{cr} was hyperuricaemic and that U_u/U_{cr} was actually lower in hyperuricaemic patients than in normouricaemic ones suggest that urate overproduction plays only a secondary role in the genesis of hyperuricaemia in SS disease.

In humans, urate cannot be further degraded. Approximately two-thirds of urate is excreted through the kidney, the rest being eliminated through the gastrointestinal tract.¹⁴ In the kidney, urate is freely filtered across the glomerulus and almost completely reabsorbed in the proximal tubules. More distally in the proximal tubule it is actively secreted and the majority of the secreted urate is subsequently reabsorbed, so that only about 12% of the filtered urate is excreted.¹⁵ Due to the great capacity for reabsorption in the proximal tubule¹⁶ the major determinants of urate clearance are tubular secretion and postsecretory reabsorption. In this study C_u/C_{cr} was significantly lower in hyperuricaemic patients than in normouricaemic patients or controls. Furthermore, an inverse correlation existed between serum urate concentrations and C_u/C_{cr} in male SS patients, suggesting that renal tubular function has a major influence on serum urate concentrations, at least in males.

Many patients with SS disease have disorders of renal tubular function which impair concentrating ability, acid excretion, and potassium excretion,^{17, 18} probably because the sickling process damages the medullary vasculature. Proteinuria in SS disease is often tubular in type¹⁹ and patients with proteinuria but little or no reduction of glomerular function have

predominantly tubular damage on renal biopsy (Morgan AG, Shah D, unpublished observations). This suggests that the proteinuric patients in our study had more severe tubular damage than the non-proteinuric ones. The lower haemoglobin values in the proteinuric patients tend to support this, since falling haemoglobin is an early indicator of renal damage in SS disease.¹⁰ Their higher serum urate concentrations and their tendency to lower C_u/C_{cr} values, which contrasts with the high C_u/C_{cr} values found in glomerular failure,²⁰ are thus further evidence to support the hypothesis that renal tubular damage is the major determinant of hyperuricaemia in SS disease.

In contrast to hyperuricaemia, gout is infrequently observed in SS disease.^{3, 4} It is possible that the prevalence of gout has been underestimated since retrospective diagnosis is difficult in a disease where self-limiting attacks of joint pain with symptom free intervals are frequent. As hyperuricaemia is common, the diagnosis will depend on joint fluid analysis for urate crystals. In a study of 56 patients with SS disease, Espinoza and coworkers did not find any case of gout during follow-up periods of 6 to 18 months.²¹ Most of our hyperuricaemic patients without proteinuria have serum urate concentrations ranging from 0.42 to 0.48 mmol/l (7.05-8.06 mg/100 ml), a concentration at which only 12% of men in the Framingham Study developed gout over a period of 12 yr.²² Long follow-up would therefore be necessary before the incidence of gouty arthritis in SS disease could be established.

In conclusion, our findings indicate that the major determinant of hyperuricaemia in SS disease is reduced renal urate clearance probably consequent upon damage to the medullary vessels and renal tubules. Urate overproduction appears to be of secondary importance but may contribute by overloading the mechanisms available for excretion. The role if any, of intestinal uricolysis in SS disease remains to be determined.

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