

# Measurement of glomerular filtration rate in homozygous sickle cell disease: a comparison of $^{51}\text{Cr}$ -EDTA clearance, creatinine clearance, serum creatinine and $\beta_2$ microglobulin

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## Abstract

**Glomerular filtration rates (GFR) were measured with  $^{51}\text{Cr}$ -EDTA in 38 patients (aged 40–75 years) with homozygous sickle cell disease and compared with serum  $\beta_2$  microglobulin concentrations in 38 patients and with creatinine clearance in 21 patients. GFR estimated with  $^{51}\text{Cr}$ -EDTA was closely correlated with single serum creatinine measurements and the inverse of serum  $\beta_2$  microglobulin. Creatinine clearance was also found to be correlated, but values were, on average, 32% below those obtained by the  $^{51}\text{Cr}$ -EDTA method, and this difference was significant.**

**It is concluded that measurements of  $\beta_2$  microglobulin, single serum creatinine, and creatinine clearance are valuable indicators of GFR in homozygous sickle cell disease. Measurement of  $\beta_2$  microglobulin was a useful and reliable method of estimating GFR from single plasma measurements and is therefore a useful means of screening the population.**

The use of serum  $\beta_2$  microglobulin, serum creatinine, and of creatinine clearance as estimators of glomerular filtration rate (GFR) has been validated against isotope methods in several populations.<sup>1-5</sup> In homozygous sickle cell disease, however, the disordered metabolism and abnormal renal tubular function<sup>6</sup> may make these techniques inappropriate. Previous studies of GFR in sickle cell disease have used creatinine clearance<sup>6-8</sup> and serum  $\beta_2$  microglobulin,<sup>9</sup> although these have not been validated against the more accurate  $^{51}\text{Cr}$ -EDTA clearance. Impaired renal function and a lowered GFR are common in older patients with sickle cell disease, and monitoring such patients requires a simple accurate method of estimating GFR. These methods were therefore compared with  $^{51}\text{Cr}$ -EDTA clearance in 38 patients aged over 40 years with sickle cell disease.

## Methods

Patients attended the sickle cell clinic at the University Hospital of the West Indies, Kingston, Jamaica. All had homozygous sickle

cell disease on the basis of standard criteria.<sup>10</sup> The patients were participants in a study of renal function in those aged over 40 years, of whom there were 158 on the clinic register. From these, 38 patients (17 men, 21 women) were selected on the basis of serum creatinine concentrations, to provide a range of predicted glomerular filtration rates, and 21 of these (eight men, 13 women) had detailed studies performed including creatinine clearance. The mean age of all subjects was 50.7 years (range 41.6–73.5 years). All were in the steady state and gave informed consent to the study.

Creatinine clearance was based on a timed and closely supervised five hour urine collection, each patient being given 400 ml water orally before the test and unlimited fluid on demand during the test. Serum and urine samples were frozen to  $-70^\circ\text{C}$  for transport to Oxford where creatinine clearance was estimated by the alkaline picrate reaction (Department of Clinical Biochemistry, John Radcliffe Hospital, Oxford).  $^{51}\text{Cr}$ -EDTA clearances were performed concurrently with creatinine clearance using the single injection method<sup>11</sup> and  $^{51}\text{Cr}$ -EDTA from Amersham Radionucleotides (Amersham UK). Serum radioactivity was counted in a multichannel spectrum analyser calibrated to the peak emission of  $^{51}\text{Cr}$  (Centre for Nuclear Sciences, University of the West Indies). Samples for serum  $\beta_2$  microglobulin were taken at the midpoint of the five hour urine collection period, frozen immediately, and transported to Oxford for estimation by radioimmunoassay (Pharmacia Diagnostics; Department of Nuclear Medicine, Radcliffe Infirmary, Oxford).

## Results

Estimates of serum  $\beta_2$  microglobulin and of  $^{51}\text{Cr}$ -EDTA clearance were performed in all 38 subjects whereas creatinine clearance and serum creatinine were measured in a subset of 21 patients (table). Creatinine clearance was linearly associated with  $^{51}\text{Cr}$ -EDTA clearance ( $r = 0.84$ ,  $p < 0.01$ ) (fig 1), although creatinine clearance seems to underestimate proportionally GFR. On average, creatinine clearance values were 24.4 ml/minute (32%) below those obtained by the isotope method, the difference between paired values being highly significant (Student's  $t$  test = 4.51,  $p < 0.0002$ ). The

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**Table** Distribution of <sup>51</sup>Cr-EDTA clearance, creatinine clearance, serum creatinine and serum β<sub>2</sub> microglobulin

Method	Number of observations	Mean	Standard deviation	Range
<sup>51</sup> Cr-EDTA (ml/minute)	38	75.9	30.2	5-132
Creatinine clearance (ml/minute)	21	51.5	24.7	5-113
β <sub>2</sub> microglobulin (mg/l)	38	3.48	2.67	1.1-15.5
Serum creatinine (μmol/l)	21	145	136	72-748

reciprocal of serum creatinine (fig 2) and of serum β<sub>2</sub> microglobulin (fig 3) showed a good correlation with <sup>51</sup>Cr-EDTA clearance (r = 0.81 and 0.79, respectively; p < 0.001 for both). No sex differences were noted in any of the comparisons (p > 0.99).

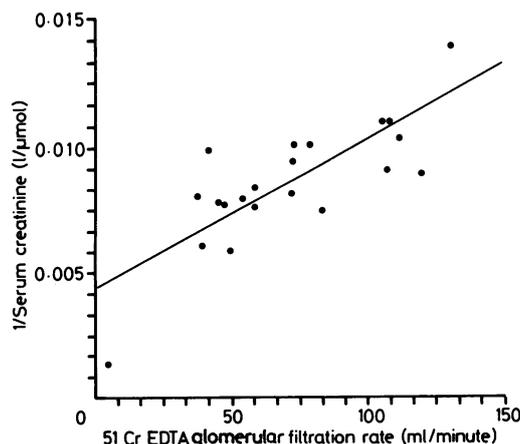
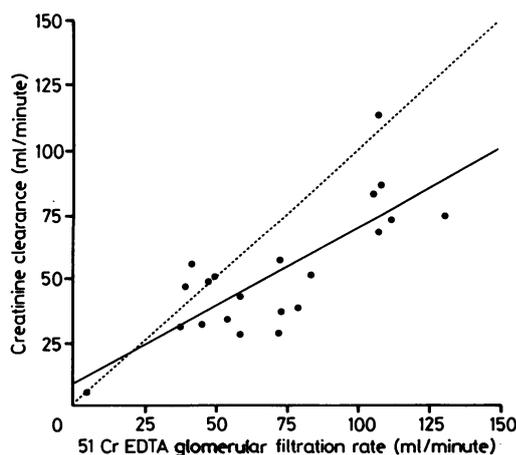
The data as presented here are not corrected for surface area. This correction was applied in analysis and no significant differences were found between the various parameters of renal function. Furthermore, in comparisons between GFR estimates such corrections cancel out mathematically.

**Discussion**

The use of single injection isotope methods for estimating GFR has been proved for a variety of isotopes including <sup>51</sup>Cr-EDTA.<sup>3,11</sup> The latter is commonly used as a reference method where inulin clearance is impractical and where larger numbers of patients need to be studied. The <sup>51</sup>Cr-EDTA method used here is believed to be robust under a variety of conditions. The major source of error occurs in the presence of moderate to severe clinical oedema, which increases the distribution time after injection, but this may be compensated for by delaying the timing of the first plasma sample. This was the case with only one patient in the study.

Endogenously produced substances are more routinely used in clinical practice and may provide good estimates of GFR. Methods based on creatinine become inaccurate when renal tubular creatinine handling is disturbed by disease and the creatinine production rate may vary with muscle mass and increased protein catabolism. Both situations may occur in patients with sickle cell disease yet studies of renal function in sickle cell disease have relied on precisely these methods. Single creatinine estimates are less reliable than creatinine

**Figure 1** Relation between <sup>51</sup>Cr-EDTA GFR and creatinine clearance in 21 patients. The solid line represents the best linear fit to the data. The dotted line represents the predicted relations—x = y.

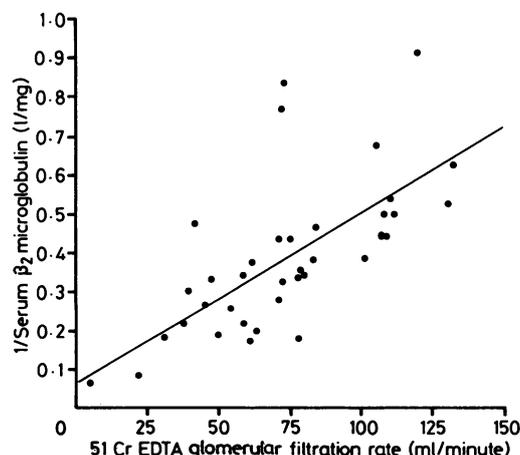


**Figure 2** Relation between <sup>51</sup>Cr-EDTA GFR and the inverse of serum creatinim in 21 patients. The solid line represents the best linear fit to the data.

clearance measurements for estimating GFR, although this study confirms that both are of use in patients with sickle cell disease. Previous studies have shown linear relations between isotope clearance methods and both creatinine clearance and the inverse of serum creatinine.<sup>1,3,4,12</sup> This study suggests that this relation is preserved in sickle cell disease and the strength of association is similar to that found by other workers.

The creatinine clearance method proportionately underestimated the GFR obtained by <sup>51</sup>Cr-EDTA. The creatinine clearance values were less than predicted in 15 of 21 (71%) of subjects and differed by a mean of 24 ml/minute (32%). One possible source of error was the short period of urine collection (five hours), but although this may contribute to more variable values of creatinine clearance, it should not produce a systematic discrepancy.

β<sub>2</sub> microglobulin is a small protein of about 100 amino acids found in association with HLA-1 molecules on all nucleated cells. The endogenous production is relatively constant and the protein is filtered and fully catabolised by the kidney.<sup>4,9,12</sup> Renal tubular disease does not lead to reappearance of the protein in the plasma. The production rate may be increased in some autoimmune or chronic inflammatory disorders,<sup>13</sup> and the increased bone marrow



**Figure 3** Relation between <sup>51</sup>Cr-EDTA GFR and the inverse of serum β<sub>2</sub> microglobulin in 38 patients. The solid line represents the best linear fit to the data.

activity in sickle cell disease could result in increased production. This possibility cannot be excluded with the available data but such increased production would tend to produce underestimates of the GFR. The  $\beta_2$  microglobulin values did not exceed those predicted by  $^{51}\text{Cr}$ -EDTA clearance (by comparison with previous studies) and in three cases the  $\beta_2$  microglobulin estimates were lower than expected. The strong linear relation between serum  $\beta_2$  microglobulin and  $^{51}\text{Cr}$ -EDTA clearance (fig 3) shows that the usefulness of  $\beta_2$  microglobulin in sickle cell disease has not been diminished, regardless of possible variation in  $\beta_2$  microglobulin production. Single estimates of serum  $\beta_2$  microglobulin have been widely used for estimation of GFR by other authors<sup>1,4,5</sup> and may be more sensitive in detecting minor reductions in GFR. The efficiency of substances used to estimate GFR may be determined from the equation:

$$\log(\text{substance}) = a \log(\text{GFR}) + c$$

in which the ideal substance has a coefficient of  $a = -1$ .<sup>12</sup> Values of  $a$  obtained for  $\beta_2$  microglobulin ( $-0.749$ ) and for serum creatinine ( $-0.588$ ) implied that the former was a more accurate estimator of GFR. Furthermore, the strength of the relation between the inverse of serum  $\beta_2$  microglobulin and GFR was similar to that found by other workers.<sup>1,4,9</sup>

If  $^{51}\text{Cr}$ -EDTA clearance is used as the reference method creatinine clearance seems to underestimate GFR in sickle cell disease. Furthermore, this method is technologically cumbersome, requiring accurately timed urine

collections, preferably over 24 hours. Measurements of serum creatinine and  $\beta_2$  microglobulin are both closely related to  $^{51}\text{Cr}$ -EDTA clearance.  $\beta_2$  microglobulin seems to offer an accurate and sensitive estimate of small reductions in GFR and its measurement in urine also allows inferences about tubular function to be made.

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