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Erythrocyte deformability in dialysed and non-dialysed uraemic patients

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Abstract. In thirty-one uraemic patients, fourteen on conservative treatment and seventeen on long-term haemodialysis, erythrocyte deformability, measured as filtration half-time in a paper filtration experiment, was studied. The two groups were comparable concerning age, sex and kidney disease.

Although the mean filtration half-time for erythrocyte suspensions was normal in non-dialysed patients there was a positive linear correlation ($P < 0{\cdot}01$) between serum creatinine and filtration half-time in this group. Filtration half-time was increased in dialysed patients, indicating impaired deformability in the latter ($P < 0{\cdot}001$). Filtration half-time showed a good inverse correlation with the packed red cell volume in the non-dialysed ($P < 0{\cdot}001$) and in the dialysed group ($P < 0{\cdot}01$).

As pre- and post-dialysis filtration half-times were the same, it appeared that the more severe uraemic state of dialysed patients was responsible for the impairment of erythrocyte deformability and not the dialysis procedure itself.

Key words. Erythrocytes, uraemia, haemodialysis, anaemia.

Introduction

Deformability of the erythrocytes is crucial for their survival in the circulation. In many types of haemolytic disorders, impaired deformability is associated with increased splenic sequestration and haemolysis [1–7], Rosenmund *et al.* [4] measured filterability as an index of erythrocyte deformability in haemodialysed patients and found a positive correlation between filtration half-time and splenic sequestration, negative correlations between filtration half-time and erythrocyte life span as well as between filtration half-time and packed red cell volume (PCV). This raised the question whether erythrocyte deformability is also altered in non-dialysed uraemic patients and how this parameter is related to the stage of renal failure. We have therefore compared erythrocyte filterability and PCV in non-dialysed uraemic patients and in patients on long-term dialysis treatment. All patients gave informed consent to the study.

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Patients

Fourteen non-dialysed patients with serum creatinine levels between 486 and 1609 μmol/l and seventeen dialysed patients with predialysis levels of creatinine ranging from 566 to 1467 μmol/l were investigated. The values in Table 1 show a pronounced anaemia in both groups. PCV, erythrocyte indices and serum phosphorus did not differ significantly; serum calcium was slightly higher in haemodialysed patients ($P < 0{\cdot}05$). Serum creatinine is not representative for the stage of renal insufficiency during haemodialysis treatment. However, the difference between creatinine levels before admission to dialysis treatment and actual creatinine levels ($P < 0{\cdot}005$) (Table 1) indicate a more severe degree of renal failure in dialysed than in non-dialysed patients although actual serum creatinine levels were similar in the two groups. None of the patients had undergone nephrectomy or splenectomy and no blood had been transfused during the 6 months before the experiment. There were no signs of acute blood loss or iron deficiency as estimated by bone marrow iron examination. Dialysis was carried out with plate dialysers (Lundia optima) or coil dialysers (Extracorporeal). The dialysate was prepared with decalcified water using the formula: Na^+ 132 mmol/l, K^+ 1·5–4·5 mmol/l, Ca^{2+} 1·75 mmol/l, Mg^{2+} 0·75 mmol/l, Cl^- 100·5 mmol/l, acetate 38 mmol/l.

Materials and Methods

Erythrocyte filterability was measured according to the method of Teitel [8] as modified by others [9, 10] and as a described here in brief.

(a) *Preparation of erythrocyte suspensions*

Blood was taken from a brachial vein in non-dialysed patients and from the arterio-venous fistula

before and immediately after treatment in dialysed patients. 18 ml of heparinized blood (50 i.u. 'Liquemin' Roche per ml) were dispensed into polystyrol tubes and centrifuged at 1150 ***g*** for 10 min. Plasma, buffy coat and a layer of 2–4 mm of erythrocytes were removed. The remaining erythrocytes were washed with an albumin containing buffer ($MgSO_4$ 1 mmol/l, NaCl 118 mmol/l, KCl 4·6 mmol/l, Na_2HPO_4 28·4 mmol/l, NaH_2PO_4 4·4 mmol/l, glucose 11·1 mmol/l, human albumin 2·5 g/l; osmolality 300 mmol/kg, pH 7·450). Centrifugation was performed at 1500 ***g*** for 5 min after the first two washings and at 2000 ***g*** for 10 min after the third washing. After each centrifugation the supernatant buffer was removed. Ultimately, a PCV of 50% was obtained by reconstitution with buffer.

(b) *Filtration device*

A glass funnel was placed vertically above a balance (Mettler, P 1210). The filter papers were prepared immediately before use as follows. A filter paper (Schleicher Schuell 589², white ribbon, Feldbach, Switzerland) with a mean pore diameter of 6·8 μm and a paper diameter of 9 cm was folded in half with its rough surface on the inside. The half circle was folded again to a quarter circle, opened, formed to a cone and put on the funnel.

(c) *Filtration of the erythrocyte suspension*

5 ml of the buffer solution kept at room temperature (21–24°C) were dispensed into the filter paper cone for prewetting and calibration of the filter paper. The time was recorded until 3 g of the solution had passed into a receiver on the balance and was noted as calibration time. At that moment a second stop watch was started. 30–40 s after completion of calibration time no more buffer flowed out of the funnel tube and the balance was readjusted to zero. In order to obtain reproducible results only filter papers with a calibration time from 30 to 35 s were used and all others rejected [10]. 1 min after completion of calibration time 2 ml of the well-mixed erythrocyte suspension, previously kept at room temperature, were put into the filter paper cone. The weight of the filtrate was recorded every 30 s until 1·2 g had passed. The filtration half-time, i.e. the time needed for 1 g ($\sim$ 1 ml) of the erythrocyte suspension to pass through the filter, could be read on semi-logarithmic paper. The assay was performed in duplicate within 3 h after blood sampling. A new filter paper was used for every assay).

The mean filtration half-time of a normal control group ($n = 17$) was 63·6 $\pm$ 7·2 s (1 SD). The coefficient of variation of twenty consecutive measurements of the same suspension was 8%.

Filtration half-time was taken as a measure of erythrocyte deformability the cells being more deformable with a short filtration half-time and more rigid with a long filtration half-time.

PCV, haemoglobin and erythrocyte indices were analysed by a Coulter Counter S.

In additional experiments mean red cell volume (MCV) and pH were measured in erythrocyte suspensions of eight non-dialysed and eight dialysed uraemic patients as well as in suspensions of eight normal individuals.

For statistical evaluation we used Student's *t*-test.

Results

The results of the filterability test are shown in Fig. 1. Patients on conservative treatment had a filtration half-time of 63·6 $\pm$ 11·4 s (mean $\pm$SD) which was within the normal range in most cases. The filtration

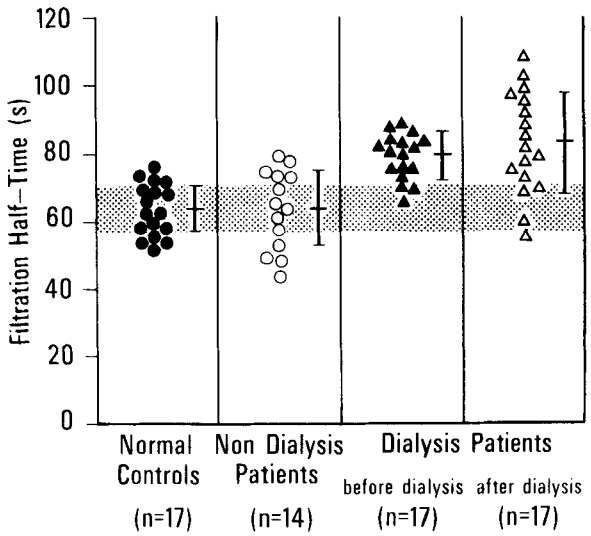

Figure 1. Results of the filterability test (mean filtration half-time $\pm$SD). The shaded area represents the normal range (mean $\pm$SD).

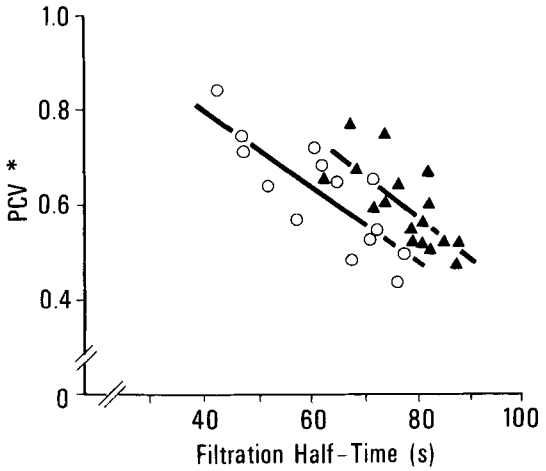

Figure 2. Correlation between filtration half-time and PCV. ○, Non-dialysis patients with corresponding regression line; ▲, dialysis patients with corresponding regression line. The two regression lines had a significant difference of intercept ($P < 0{\cdot}01$), but the difference of slope was not significant. *PCV ($\bar{x}$ 3 months) is expressed as the fraction of the normal mean value in relation to sex, thus 1·0 in the graph corresponds to a PCV of 0·43 for women and 0·47 for men.

Table 1. Clinical and laboratory details

	Non-dialysis patients ($n=14$)	Dialysis patients ($n=17$)	Normal range
Age (years)	39–68	29–64	
Sex (m/f)	4/10	6/11	
Renal disease			
Chronic pyelonephritis and interstitial nephritis	6	8	
Chronic glomerulonephritis	2	5	
Polycystic kidneys	3	3	
Renal failure of unknown origin	3	1	
PCV/(normal mean PCV)*	0·62 ± 0·12	0·58 ± 0·07	0·88–1·12
MCV (fl)	88·6 ± 6·1	90·7 ± 6·5	80–102
MCH (fmol)	1·8 ± 0·2	1·8 ± 0·2	1·7–2·0
MCHC (mmol/l)	20·0 ± 0·7	19·8 ± 0·9	18·6–21·7
Reticulocytes (g/l)	41 ± 23	34 ± 19	15–108
Serum creatinine (μmol/l)	919 ± 265	1193 ± 256‡ 972 ± 194†	44–115
Serum urea nitrogen (mmol/l)	34·5 ± 9·2	30·2 ± 7·4	2·9–7·5
Serum calcium (mmol/l)	2·3 ± 0·4	2·5 ± 0·3	2·3–2·7
Serum phosphorus (mmol/l)	1·6 ± 0·5	1·7 ± 0·4	0·5–1·5

The laboratory data represents mean ± SD of three determinations during the three preceding months, actual values included. For dialysis patients, values before dialysis are presented with one exception (see below ‡).

* PCV is expressed as the fraction of the usual mean value in relation to sex, thus 1·0 corresponds to a PCV of 0·43 for women and 0·47 for men.

† Actual serum creatinine level on dialysis treatment (before dialysis).

‡ Serum creatinine level before admission to the haemodialysis treatment programme.

half-time of dialysed patients was 78·6 ± 7·2 s, which was significantly prolonged in comparison to the control group (63·6 ± 7·2 s, $P<0·001$) as well as to the non-dialysed patients ($P<0·001$). There was no significant difference between mean filtration half-time before (78·6 ± 7·2 s) and after dialysis (82·2 ± 15 s).

Fig. 2 demonstrates the inverse correlation of the filtration half-time with the PCV in the non-dialysed group ($r=-0·81$, $P<0·001$, $n=14$) and in the dialysed group ($r=-0·71$, $P<0·01$, $n=17$). At the same filtration half-time, dialysed patients had a higher PCV than non-dialysed patients ($P<0·01$). There was no correlation between filterability and PCV in our normal control group ($n=17$), but this could not be expected in the narrow range of PCV values. There was a positive correlation between creatinine level and filtration half-time in the non-dialysed patients ($r=0·73$, $P<0·01$, $n=14$), whereas in the dialysed patients creatinine levels before and after dialysis did not correlate with the filtration half-time. Between serum calcium and filtration half-time there was a slight inverse correlation in the non-dialysed group ($r=-0·61$, $P<0·05$, $n=14$) and no correlation in the dialysed group. Serum urea was the same in the two groups (Table 1) and there was no correlation between serum urea and filtration half-time in the dialysed and the non-dialysed uraemic groups.

The erythrocyte indices and the reticulocyte count as measured in whole blood of the patients are presented in Table 1. Investigations of MCV and pH in erythrocyte suspensions gave the following results. The MCV was 92·6 ± 5·9 fl in non-dialysed uraemic patients ($n=8$), 89·7 ± 5·9 fl in dialysed patients before dialysis and 89·0 ± 5·6 fl after dialysis ($n=8$). In suspensions of normal controls the MCV was 89·4 ± 3·2 fl ($n=8$). The pH in the same suspensions was 7·24 ± 0·09 in non-dialysed patients ($n=8$), 7·32 ± 0·03 in dialysed patients before dialysis and 7·33 ± 0·02 after dialysis ($n=8$). In control suspensions the pH was 7·28 ± 0·02 ($n=8$).

Coombs tests were not performed for the purpose of this study but had been performed in eleven haemodialysed patients, some more than 6 months before the study and the others after the study for routine blood testing. All the direct Coombs tests were negative as were indirect Coombs tests with the erythrocytes of blood donors.

Discussion

Erythrocyte deformability was found to be impaired in various haemolytic disorders. In these conditions erythrocyte life span and erythrocyte deformability as measured in paper filtration experiments [7] are positively correlated. This was also found by Rosenmund and co-workers for patients on chronic haemodialysis treatment [4]. Our results confirm the findings of the latter authors, i.e. prolonged filtration half-time as well as the negative correlation between filtration half-time and PCV of patients on haemodialysis treatment.

In the present study a similar correlation was found for the non-dialysed group. However, the mean filtration half-time was normal. The better deformability of erythrocytes of non-dialysed as compared to dialysed uraemic patients may in part explain the longer life span of erythrocytes in non-dialysed patients as observed by others [11–13].

The reticulocyte counts were low in both patient groups. It can therefore be concluded that haemolysis was not severe in these patients. The reticulocyte values alone do not allow any conclusions about whether erythrocyte life span was different in the two groups because in mild to moderate degrees of haemolysis there is no correlation between reticulocytes and red cell life span [14]. The finding, however, of decreased paper filterability of erythrocytes in our dialysed group suggests that these erythrocytes had a shorter potential life span than those of non-dialysed patients.

Although actual serum creatinine levels of the two groups were the same, the significantly higher serum creatinine before admission to the dialysis treatment indicates a more severe stage of renal failure in dialysed patients as compared to the non-dialysed group. Moreover, according to the natural history of underlining renal diseases a progression of renal failure during dialysis treatment must be assumed.

In the earlier stages of renal failure (i.e. in non-dialysed patients) erythrocyte deformability decreased as serum creatinine increased as demonstrated by the positive correlation between creatinine and filtration half-time. The mean filtration half-time, however, remained within the normal range. In contrast, patients on dialysis therapy showed significantly impaired deformability, but there was no correlation between creatinine level and filtration half-time. This is not surprising because serum creatinine is not a reliable parameter of renal function on dialysis treatment. The lack of a difference between filtration half-time before and after dialysis makes it unlikely that the impairment of deformability was due to the dialysis procedure.

There was no difference between the MCV in erythrocyte suspensions of controls, non-dialysed and dialysed patients as assessed in complementary experiments. The pH difference in erythrocyte suspensions were very small and cannot therefore explain the substantial differences of filtration half-time [15].

As none of the laboratory investigations explained the difference of filtration half-time, and as filtration half-time was the same before and after dialysis, it may be concluded that a more severe uraemic state was responsible for the worse deformability of erythrocytes in dialysed patients.

The hitherto available dialysers do not clear the blood with the same efficacy as the kidneys and, as a consequence, many disorders associated with renal insufficiency persist or progress despite dialysis treatment, such as abnormalities attributed to retained substances [16], alterations of lipid metabolism [17–19] and endocrine disorders [20]. To what extent such abnormalities may contribute to the impairment of deformability remains unknown at present, but our results suggests that in severe renal insufficiency the erythrocyte may be a target for these disorders. As long as more reliable guidelines for dialysis treatment are not available the assessment of erythrocyte deformability might be a valuable help for the control of the efficacy of haemodialysis.

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