

QUANTITATION OF URINARY RED BLOOD CELLS BY PHASE-CONTRAST MICROSCOPY: ITS RELATIONSHIP TO SEVERITY OF GLOMERULAR DAMAGE

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SYNOPSIS

The present study utilised phase-contrast microscopy (P-CM) to quantitate and characterise the morphology of the urinary red blood cells (RBCs) excreted in microscopic range by 50 consecutive patients admitted for renal biopsy and correlated the urinary findings with various clinicopathologic parameters.

All proliferative glomerulonephritis (GN) patients (41/50) excreted abnormal numbers of dysmorphic urinary RBCs and the only patient with normal renal histology had numerous isomorphic RBCs. Nephrotic patients (8/50) excreted dysmorphic RBCs in normal range. GN patients excreting 80,000 RBCs/ml were more likely to have underlying glomerular crescents ($X^2 = 9.95$, $p = 0.005$) or global sclerosis ($X^2 = 5.56$, $p = 0.02$). The number of urinary RBCs excreted did not correlate with segmental sclerosis, serum creatinine or proteinuria. 17/20 (85%) who excreted mixed pattern of dysmorphic (8000/ml) and isomorphic (5-30%) RBCs had underlying IgA GN. Oral Frusemide 40 mg given to 12 patients increased the % of isomorphic RBCs from 11.8 ± 9.9 (mean \pm SD) to 83.1 ± 9.4 ($t = 17.25$, $p = 0.001$) 1.5 hours later.

In conclusion, P-MC of urinary sediment is useful in confirming haematuria and localising its source. Increased excretion of dysmorphic RBCs (> 80,000/ml) is more likely to indicate underlying glomerular crescents or global sclerosis. Oral Frusemide should not be served before urinary P-CM because of its significant effect on morphologic transformation of urinary RBCs.

INTRODUCTION

Phase-contrast microscopy (P-CM) of urinary sediment has been shown to be useful in localising the source of haematuria based on the morphology of urinary red blood cells (RBCs) (1). Bleeding from the glomerulus is characterised by dysmorphic RBCs (> 8000 cells/ml) in the urine whereas the RBCs in non-glomerular bleeding are isomorphic in nature. The precise mechanism(s) of dysmorphism of urinary RBCs is not defined although osmotic change of the red-cell membrane during passage through the distal tubules has been suggested as a possibility (2).

The increased likelihood of glomerular crescents in IgA nephropathy patients who had macroscopic haematuria has been shown by some (3) and not by others (4,5). It is uncertain if high RBC excretion in the microscopic range is correlated with active glomerular lesions such as crescents. The present study examined the value of quantitating microhaematuria by P-CM in predicting the severity of glomerular damage in various forms of glomerulonephritis (GN) and the effect of oral Frusemide which acts in the ascending loop of Henle on the morphology of urinary RBCs in GN patients.

METHODS AND MATERIALS

Between July and October 1985, urine samples were obtained from 50 consecutive patients admitted to the Renal Unit, Singapore General Hospital for renal biopsy. Two fresh 10-ml aliquots of urine were sent for P-CM and routine microscopy respectively. For P-CM, a 10-ml urine sample was centrifuged for 10 minutes at 2000 rpm at 25 C. From this, 9.5 ml of the supernatant was discarded and the resuspended sediment was examined in a Fuchs-Rosenthal chamber, using Olympus BH microscope equipped with positive phase-contrast illumination. For routine microscopy, 10 ml of urine was centrifuged for 7 minutes, the supernatant was poured off, and the sediment was examined under high-powder magnification ($\times 400$).

Significant glomerular haematuria as defined by P-CM is ≥ 8000 dysmorphic RBCs/ml (6). The upper limit for RBCs in the urine for normal subjects by routine microscopy is 5/high power field (HPF).

Percutaneous renal biopsy was performed by a Tru-Cut needle (Travenol, USA). Renal tissue was fixed in Dubosq-Brazil solution and 2- μ m sections were cut and stained with haematoxylin and eosin, periodic acid-Schiff and silver-Massons trichrome. Immuno-

fluorescent staining was performed on 6- μ m cryostat sections using FITC-conjugated anti-human IgA, IgG, IgM, C1q, C3 and fibrinogen (Behring, FRG). Glomerular lesions such as glomerulosclerosis (global, segmental) and crescents were counted and expressed as percentage of total glomeruli per tissue section.

The effect of Frusemide on dysmorphism of urinary RBCs was studied. Oral Frusemide 40 mg was served to 12 patients prior to renal biopsy. Urine samples collected before, 1, 1.5 and 2 hours after administration of Frusemide were examined by P-CM. The percentage of isomorphic RBCs in the urinary sediment of each sample was recorded.

Statistical analyses were performed using Student's t test, chi-square test and linear correlation coefficients. All values were expressed as mean \pm SD.

RESULTS

The mean age of the 50 patients (M 26, F 24) was 27.0 ± 11.7 years. Forty-one patients had significant haematuria (≥ 8000 cells/ml) as confirmed by P-CM. However, routine urine microscopy only documented haematuria (≥ 5 /HPF) in 80% (33/41) of these cases. On the whole, the correlation between the two methods in detecting haematuria was good ($r = 0.687$, $P < 0.001$) (Fig. 1).

All the 41 patients who had excreted dysmorphic RBCs (Fig. 2) in excess of 8000 cells/ml were proved to have proliferative GN by biopsy. Eight nephrotic patients had ≥ 8000 /ml of dysmorphic RBCs and their biopsies showed non-proliferative lesions. The only patient whose renal histology was normal had excreted numerous isomorphic (Fig. 3) and also dysmorphic (5000 cells/ml) RBCs in the urine. Arteriographic and urological investigation failed to localise the source of bleeding in this patient.

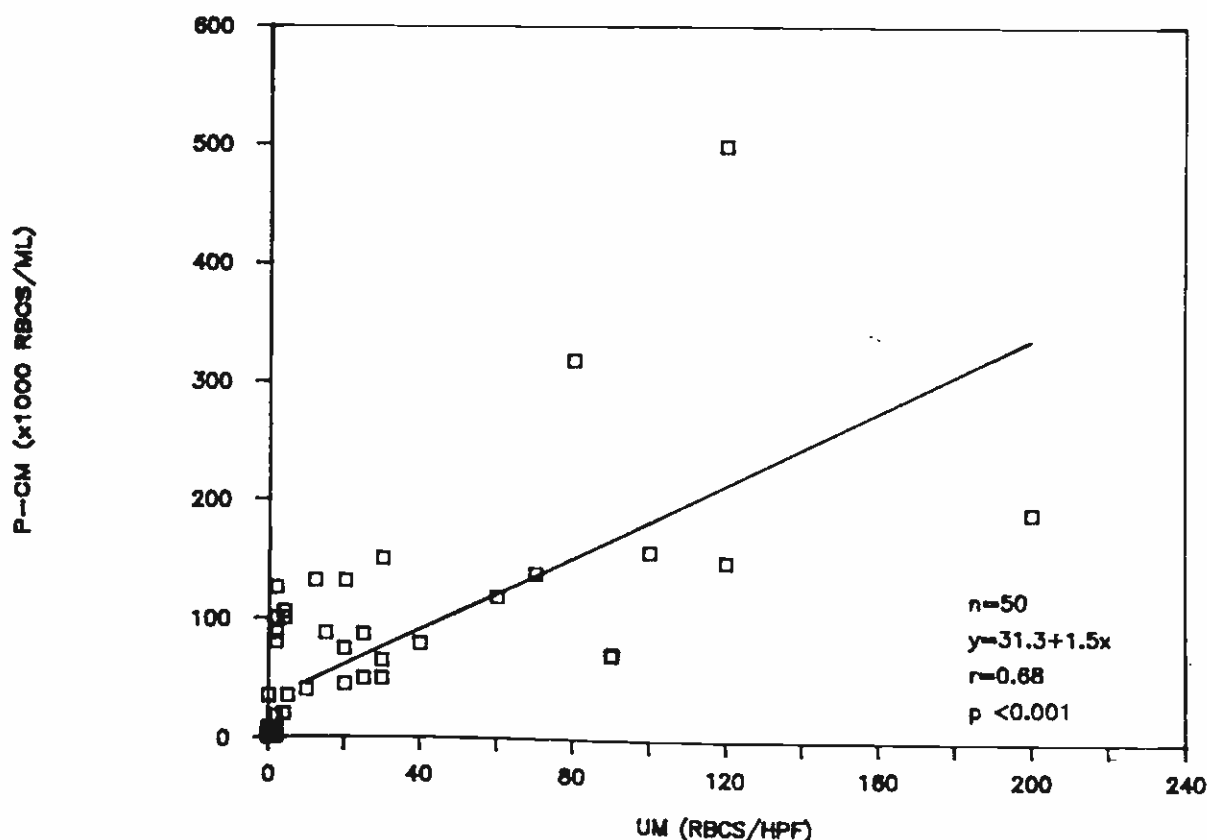


Fig 1 Correlation between urinary RBC counts by P-CM (RBCs/ml) and urine microscopy (UM) expressed as RBCs/HPF.

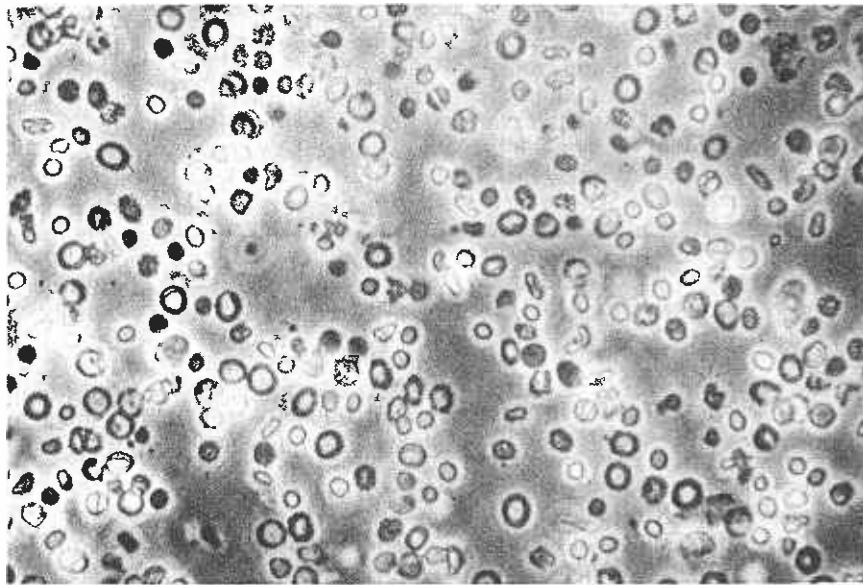


Fig. 2 Dysmorphic urinary RBCs, x 400 magnification.

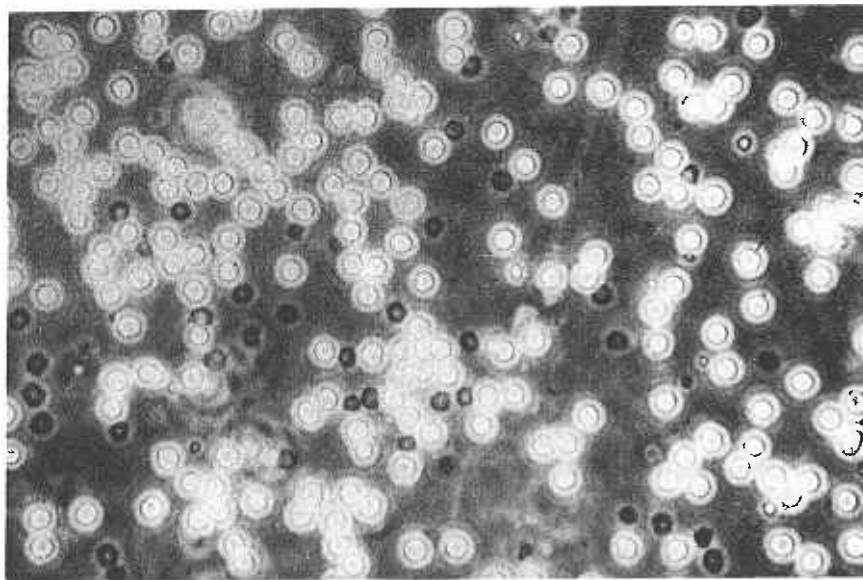


Fig. 3 Isomorphic urinary RBCs, x 400 magnification.

The heterogeneity of the underlying GN in the 49 patients is illustrated as follows: IgA nephropathy (N=29), lupus nephritis (N=9), focal global sclerosis (N=3), focal and segmental glomerulosclerosis (N=4), diffuse mesangial proliferative GN (N=1), focal mesangial proliferative GN (N=1), minimal change disease (N=1) and diffuse sclerosing GN (N=1).

Moderate haematuria was arbitrarily defined as 80,000 dysmorphic cells/ml ($\times 10$ normal). Those GN patients who excreted more than 80,000 dysmorphic cells/ml were more likely to have underlying glomerular crescents ($X^2 = 9.95$, $P = 0.005$) or global glomerulosclerosis ($X^2 = 5.56$, $P = 0.02$). However, the number of urinary RBCs did not correlate with the percentage of segmental glomerulosclerosis, serum creatinine, creatinine clearance or proteinuria in the 49 GN patients.

17/20 (85%) patients excreting a mixture of dysmorphic (> 8000 cells/ml) and 5–30% isomorphic RBCs were found to have biopsy-proven IgA nephropathy. This is significant when compared to the diagnosis of IgA nephropathy in only 40% of those patients who excreted solely dysmorphic RBCs in the urine ($X^2 = 4.46$, $P = 0.05$).

Oral administration of Frusemide 40 mg to 12 patients resulted in significant change in the proportions of isomorphic RBCs in the urine. The change was maximal at 1.5 hours after medication when the % of isomorphic RBCs was 83.1 ± 9.4 as compared to the baseline % of 11.8 ± 9.9 ($t = 17.25$, $P = 0.001$).

DISCUSSION

Our study confirms the increased sensitivity of P-CM over routine microscopy of the urine sediment in

detecting significant haematuria. This is not surprising as the HPF method is not truly quantitative because of the varying volume under the coverslip. In addition, the magnification used is often uncertain and if the excretion-rates of urinary RBCs are low, numerous HPFs must be examined to avoid missing abnormal values. Although the HPF method missed 20% of our patients who had significant haematuria confirmed by P-CM, the detection rates between the two methods correlated well.

Previous workers (1,6) have described the morphological abnormalities of urinary RBCs in GN. Our findings substantiate the predictive power of P-CM of urinary-RBC morphology in localising the site of haematuria. All our patients who have proliferative GN had excreted 8000 dysmorphic RBCs/ml. Nephrotic syndrome secondary to non-proliferative GN such as focal glomerulosclerosis, focal and segmental glomerulosclerosis or minimal change disease was associated with lower numbers of urinary RBCs (8000/ml) as in 8 of our patients. In this group of patients, urinary P-CM is less helpful in confirming glomerular bleeding but the presence of nephrotic syndrome should point to a glomerular problem. One patient who had normal renal histology confirmed by biopsy excreted numerous isomorphic and 5000/ml of dysmorphic RBCs. The source of bleeding in this patient was most likely in the lower tract in spite of negative arteriographic and urological findings. The presence of 5000/ml of dysmorphic RBCs is of no significance as RBCs seen in healthy subjects are dysmorphic and may be present in numbers up to 8000/ml (6).

In spite of the heterogeneous nature of the underlying GN in our patients, those who had moderate haematuria (80,000/ml) were more likely to have glomerular crescents or global sclerosis. The association between high urinary RBC counts and active glomerular disease with crescentic change has been reported in IgA nephropathy (3). Crescentic lesions accompanying various forms of GN are thought to indicate poor prognosis (7,8). The correlation between urinary RBC excretion and crescents may be explained by escape of RBCs through breaks in glomerular basement membrane associated with crescentic lesions (9,10,11). The higher prevalence of global glomerulosclerosis may represent end-stage damage of previous glomerular lesions such as crescents or segmental sclerosis. Other clinicopathological data such as segmental sclerosis, serum creatinine, creatinine clearance or proteinuria did not correlate with urinary RBC counts. It appears that high dysmorphic RBC excretion may be a useful marker of underlying crescents but this needs to be confirmed in larger studies.

Excretion of a mixture of dysmorphic (8000 cells/ml) and 5–30% of isomorphic RBCs was noted in 20 patients. Out of these, 17 (80%) were found to have biopsy-proven IgA nephropathy. This is significant when compared to the diagnosis of IgA nephropathy in only 40% of all those patients who excreted exclusively dysmorphic RBCs. The source of the isomorphic cells is presumably non-glomerular but urological investigations in a number of these patients were unhelpful. It has been suggested by some workers (1) that IgA deposits in mucosal vessels of the urinary tract may contribute to bleeding. We are currently looking into the sensitivity and specificity of this mixed urinary-RBC pattern in diagnosing IgA nephropathy in a large patient population.

The precise mechanism(s) of dysmorphism of urinary RBCs is not known. Previous workers (2) have

attributed the RBC-membrane deformity to the sequential changes of osmolality of tubular fluids particularly the hypotonicity at the ascending loop of Henle. The use of oral loop-diuretic, Frusemide, in our patients increased significantly the % of isomorphic RBCs from 11.8 ± 9.9 to 83.1 ± 9.4 in the urinary sediment 1.5 hours later. Other workers (2) have documented reversal of isomorphism to dysmorphism 6 hours later. This suggests strongly the critical role of osmotic environment in the tubule in inducing RBC dysmorphism. It is recommended that loop-diuretics should not be administered to patients within 6 hours prior to urinary P-CM.

In conclusion, P-CM of the urinary sediment appears to be a useful diagnostic technique in localising the site of haematuria. Those patients excreting 80,000/ml of dysmorphic RBCs are more likely to have underlying glomerular crescents or global sclerosis. Significantly high % of IgA nephropathy patients excreted mixed pattern of glomerular and non-glomerular RBCs. Oral Frusemide has a significant effect on morphological transformation of urinary RBCs via its osmotic influence in the tubule and the effect is maximal at 1.5 hours after medication.

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