Original Articles

URINARY RED CELL MORPHOLOGY TO DETECT SITE OF HEMATURIA

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ABSTRACT

We studied the urinary RBC morphology in 87 consecutive cases of significant hematuria by 3 commonly used methods: (a) light microscopy of the unstained urinary sediment; (b) phase contrast microscopy of the unstained urinary sediment; and (c) Wright's staining of the urinary sediment, in order to compare the sensitivity of these methods in detecting dysmorphic RBCs and thus predicting the site of hematuria. The clinical data and the relevant investigations were made available after the morphology of RBCs in the urine was identified. Out of the 87 patients, 45 had a glomerular and 42 had a nonglomerular cause o hematuria.

Phase contrast microscopy showed a sensitivity of 91.1%, Wright's stain of 82.2% and light microscopy of 66.7% in detecting a glomerular source of hematuria. Nonglomerular hematuria could be detected in 92.9% cases by each of the 3 methods.

It is concluded that phase contrast microscopy is most sensitive for the detection of dysmorphic RBCs in the urine, Wright's stain nearly as sensitive whilst light microscopy of the unstained sediment is least sensitive. Urinary RBC morphology is a useful adjunct in the diagnosis of hematuria and saves the patients from unnecessary investigations.

Key words: *Hematuria, Glomerular, Microscopy.*

In 1979 Birch and Fairley using phase contrast microscopy demonstrated that abnormal morphology of red cells in urine can differentiate glomerular from nonglomerular hematuria in adults(l). According to them, red cells coming through the glomerulus were dysmorphic whereas, those from the lower urinary tract were eumorphic. Subsequently, usefulness and sensitivity of this method in children was confirmed by several workers(2-4). Later on examination of urinary sediment stained by Wright's stain under light microscope was reported to be as good as phase contrast microscopy with the added advantages of easy availability, being less expensive as well as providing permanent record(5-7). There are no studies comparing these three methods simultaneously in children. Hence we designed this study to assess the sensitivity of (*i*) phase contrast microscopy (PCM), (*ii*) Wright's staining of urinary sediment (WS), and *(iii)* simple light microscopy (LM) of unstained sediment as a preliminary test to localize the site of hematuria and to guide further line of investigations.

Material and Methods

Urinary red cell morphology of 87 con-

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secutive children with significant hematuria(8) were studied blindly by light microscopy of unstained standard urine sediment (LM), light microscopy of sediment using Wright's stain (WS) by modification of method of Chang(5) and phase contrast microscopy (PCM) of unstained sediment(9,10).

Fresh urine samples with specific gravity (SG) 1010 were examined within 60-90 min of voiding. A minimum of 100 RBCs were examined by each technique. If more than 20% of red cells showed dysmorphism, glomerular basis of hematuria was suspected *(Fig. 1)*. Eumorphic red cells which resembled circulating RBCs in peripheral blood indicated non glomerular hematuria *(Fig. 2)*.

In each case, a final diagnosis was made by standard clinical, biochemical, bacteriological, immunological, radiological and histopathological investigations and correlated with the type of hematuria. Twelve cases of acute glomerulonephritis (AGN) were followed up with serial urine examinations for 4 weeks to detect changes in urinary erythrocyte count and percentage of dysmorphic red cells in urine. The specificity and sensitivity of the three methods were compared statistically.

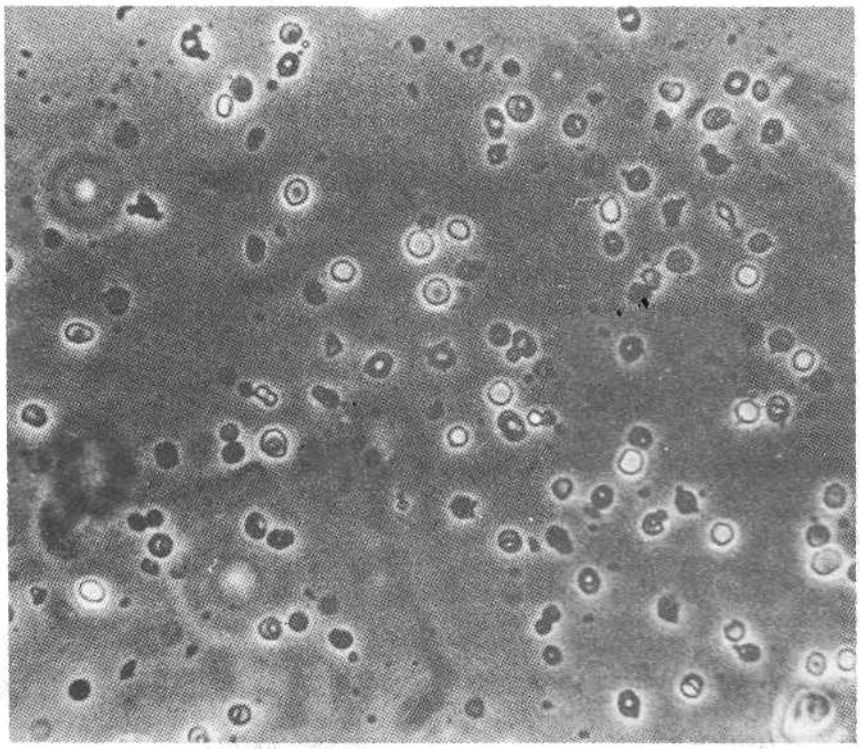

Fig. 1. Dysmorphic RBCs indicating glomerular hematuria by phase contrast microscopy (distorted, small, buds and blebs).

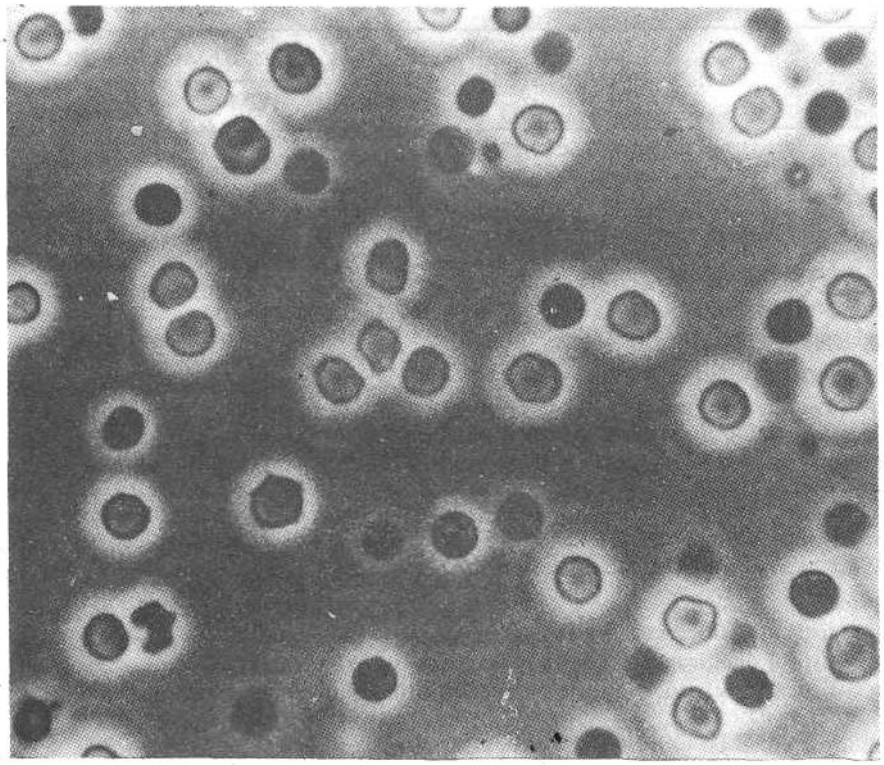

Fig. 2. Eumorphic RBCs indicating nonglomerular hematuria by phase contrast microscopy (Uniform size, RBCs "ghosts").

Results

Out of 87 children between 2 days-15 ears of age with significant hematuria, 50 were boys and 37 girls. In 45, glomerular bleeding was diagnosed by clinical biochemical and immunological parameters and in 13, renal histopathology was available for diagnosis *(Table I);* 42 had nonglomerular causes as diagnosed by standard clinical, biochemical, bacteriological and radiological investigations *(Table II).* To evaluate efficacy of each method, number of glomerular (dysmorphic RBCs) and nonglomerular (eumorphic RBCs) hematuria detected by each method were compared statistically *(Tables I & II)* and it was found that PCM had a sensitivity of 91.2%; WS of 82.3% and LM of 66.6% in diagnosis of glomerular bleeding. Nonglomerular hematuria was detected in 39/42 (93%) cases by each method.

Using standard error of difference between two proportions, it was noted that there was no significant difference between percentage of dysmorphism obtained by PCM and WS (76 ± 17.5 *vs* 65 ± 16) but there were significant differences between

TABLE I-*Correlation of Urinary Dysmorphic ROCs with Glomerular Disease.*

Etiology	No.	Dysmorphic RBCs by		
		PCM	WS	LM
Acute glomerulonephritis	30	28	25	20
Lupus nephritis	5-	4	4	3
Hemolytic uremic syndrome	2	1	1	1
Henoch Schonlein Purpura	2-	2	2	1
Membranoproliferative glomerulonephritis	2-	2	1	1
Chronic glnmerulnn'pbriti, IgA nephropathy	4-	4	4	4
Mesangioproliferative glomerulonephritis	(1 each)			
Benign hematuria (normal biopsy)				
Total	45	41 (91.2%)	37 (82.8%)	30 (66.7%)

* Biopsied.

High statistical significance when PCM compared with LM $p < 0.001$
High statistical significance when PCM compared with WS $p < 0.001$
High statistical significance when WS compared with LM $p < 0.001$

TABLE II-*Correlation of Nonglomerular Diseases with Eumorphic ROCs.*

Etiology	No.	Eumorphic RBCs in urine using		
		PCM	WS	LM
UTI	18	17	16	17
Postsurgical	8	8	8	8
Calculi	6	5	6	5
Hypercalciuria	3	2	2	3
Renal tuberculosis	1	1	1	0
Leukemia, Scurvy, Hemophilia, Pyonephrosis, Cyclophosphamide induced viral infection	6 (one each)	6	6	6
Total	42	39 (93%)	39 (93%)	39 (93%)

results obtained when PCM and WS were compared with LM (49 ± 20) ($p < 0.001$). RBC casts were seen by PCM in 20/45 and by LM only in *9/45 cases* of glomerular hematuria. In 13/25 cases there were no casts/proteinuria noted.

False Negative Cases

Of the false negative cases 4 were by PCM, 8 by WS and 15 by LM. Out of 30 patients of acute glomerulonephritis, eumorphic RBCs were seen in 2 by PCM in 5 by WS and in 10 cases by LM. One case each of lupus and hemolytic uremic syndrome (HUS) revealed eumorphic RBCs by PCM, 3 additional cases by WS and 5 by LM with false -ve results were noted *(Table I).*

False Positive Cases: There were 3 false positive cases by each method. Non glomerular disorders with significant dysmorphism were noted in cases of calculi, urinary tract infection (UTI), idiopathic hypercalciuria and renal tuberculosis, which is also reported by other authors(2,5,10). Progression of dysmorphism was seen in 10/12 cases of acute glomerulonephritis on weekly follow up over a 4 week period. The data was analyzed to decide the predictive value of the percentage of dysmorphic RBCs for diagnosis of glomerular hematuria. *Table III* compares the yield of 20%, 40% and 80% of dysmorphic RBCs by PCM. Specificity was 100% when 40% and 80% were taken as cut off points, whilst the sensitivity was 88.8% and 42.2%, respectively. At 20% of dysmorphism, the sensitivity (91.1%) and specificity (92.86%) were suitable for differentiating glomerular from nonglomerular hematuria *(Table III).*

TABLE III-*Sensitivity and Predictive Value of Percentage of Dysmorphic RBCs on Phase Contrast Microscopy*

Dysmorphic RBCs(%)	Sensitivity (%)	Specificity *(%)*
20	91.1	92.86
40	88.8	100
80	42.2	100

Discussion

There are a few reports of RBC dysmorphism used for detecting the site of hematuria in pediatric patients(2,3,4,7,ll), but none from India. In our country with limited resources and lack of facilities for advanced investigations it is imperative to have a simple test to decide which case of hematuria should be subjected to the elaborate, expensive and invasive tests. From our study we could predict the site of hematuria in 92% patients using PCM, 88% using Wright's stain and 80% using plain light microscopy, but there was greater variation in the sensitivity of detection of glomerular hematuria (91%) by PCM, 82% by WS and 67% by light microscopy).

Twenty five of the forty five patients with glomerular disease did not show casts and in 13 out of these 25, there was no proteinuria. Hence, 13/45 patients with glomerular disease might have been subjected to urologic/radiologic procedures such as IVP/ cystoscopy. However, on basis of red cell morphology it is possible to channelize this group towards specific investigations and if required renal biopsy; otherwise cases of IgA nephropathy, hereditary nephritis, *etc.* may be missed.

High sensitivity of PCM in detection of glomerular hematuria in the range of 92-97% has been reported by various workers(l,2,3,4,10) and compares well with 91.2% observed by us *(Table IV).* Chang reported 90-100% sensitivity using Wright's Stain(5), but we observed only 82% sensitivity with Wright's stain in detection of glomerular hematuria.

The controversial issue is the percentage of dysmorphic RBCs for detection of

TABLE IV–*Sensitivity Reported by Different Workers Using Various Percentage of Dysmorphism*

Study	Dysmorphism (%)	Sensitivity (%)	Population
Rizzoni(2)	40	97	Children
Fassett(10)	20	96	Adults
Birch(1)	80	95	Adults
Stapelton(3)	10	92	Children
Ours	20	91	Children

glomerular hematuria. The percentage of dysmorphic RBCs required for the diagnosis of glomerular hematuria has varied widely in different studies ranging from 80% to as low as 10% *(Table IV);* and most of the studies have been in adults(2,3).

In our study the presence of more than 40% dysmorphic RBCs has a 100% predictive value for glomerular hematuria but is likely to miss 11.2% cases of glomerular hematuria that have lesser degrees of dysmorphism. On the other hand using a cut off value of 20% of dysmorphism has an increased sensitivity, with slight fall in specificity to 93%. Twenty per cent dysmorphic RBCs could, therefore, be used as a cut off value to screen for glomerular hematuria.

In conclusion, this study clearly shows that whenever hematuria is not accompanied by typical clinical features of glomerulonephritis (edema, hypertension) and if there are no casts in urine, dysmorphic red cells indicate glomerular hematuria if 20% or more of urinary red cells are dysmorphic. In case of asymptomatic isolated hematuria, if dysmorphic RBCs are detected and ultrasonography has ruled out calculous disease, further investigations (which may include kidney biopsy) for diagnosis of glomerular disease are required. On the other hand if eumorphic RBCs are found then investigations should include urine culture, calcium excretion studies, renal ultrasound, voiding cystourethrogram and urologic consultation for nonglomerular diseases. The most sensitive method to detect glomerular/nonglomerular hematuria at present is phase contrast microscopy, but the cost and need for training makes it exclusive and Wright's stain which is easily accessible may be used for routine purpose of detection of dysmorphic red cells in urine.

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