Original Article

Differentiating glomerular from non-glomerular hematuria: role of urinary albumin-total protein ratio

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ABSTRACT

***Background**: Hematuria is one of the most common and early signs of diseases related to genitourinary system and can be classified as either glomerular or non-glomerular in origin. Percentage of dysmorphic RBC (%dRBC) in urine has been in practice as a diagnostic tool for differentiating glomerular from non-glomerular hematuria. Recent studies indicate that, urinary albumin-total protein ratio (uAPR) can also be used as a diagnostic tool in this regard. This study aimed to evaluate the sensitivity and specificity of uAPR as a diagnostic tool for detecting glomerular hematuria in comparison to %dRBC in urine.*

***Methods:** This cross-sectional study enrolled 96 patients with hematuria. Fresh urine samples were collected from each subject to determine the %dRBC and to calculate uAPR. Receiver operating characteristic curve analysis was done on these results to evaluate the sensitivity and specificity of uAPR and %dRBC in differentiating glomerular from non-glomerular hematuria.*

***Results**: uAPR and %dRBC were significantly ($p<0.001$) higher among patients with glomerular hematuria than non-glomerular hematuria. At the cutoff value of 0.57 mg/mg, uAPR showed sensitivity of 81.8% and specificity of 95.5%. At the cutoff value of 22.5%, %dRBC showed sensitivity of 54.5% and specificity of 86.4%.*

***Conclusion**: uAPR has higher sensitivity and specificity than %dRBC in differentiating glomerular from non-glomerular hematuria and can be used as a diagnostic tool.*

***Key words**: dysmorphic red cells, hematuria, phase-contrast microscopy.*

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INTRODUCTION

Hematuria is widely associated with conditions involving genitourinary system including kidneys, ureters, prostate gland, bladder and urethra requiring immediate diagnosis.[1] Presence of three or more red blood cells on a single, properly collected, non-contaminated urinalysis without evidence of infection is considered as clinically significant microscopic hematuria.[2] The prevalence of asymptomatic microscopic hematuria ranges from 0.19-21%.[3] Urinary lithiasis, glomerular and tubular diseases, neoplasia or infection of the kidney and lower urinary tract and rupture of capillary blood vessels act as major cause of hematuria.[4] Depending on the source of bleeding, hematuria can be classified as either glomerular or non-glomerular. Glomerular hematuria is characterized by dysmorphic red blood cells (dRBC) whereas non-glomerular hematuria is characterized by isomorphic erythrocytes.[5] Clinical presentation of glomerular diseases ranges from asymptomatic presentation to end stage renal disease (ESRD).[6] Glomerular diseases are one of the leading causes of ESRD globally.[7] Each year around 13,000 new patients develop ESRD in Bangladesh and glomerulonephritis is the cause for 40% of these ESRD cases.[8]

At present, a number of diagnostic tests, many of them invasive, are performed in patients with hematuria leading to increased healthcare expenses and discomfort.[9] The ability to differentiate glomerular from non-glomerular hematuria early on, leads to faster diagnosis, minimizing the expenses and discomfort to the patient.[10]

Although phase-contrast microscopy (PCM) to determine the percentage of dysmorphic red blood cells (%dRBC) in urine is a well-established technique to differentiate glomerular and non-glomerular hematuria, this investigation is time consuming and the sensitivity and specificity may vary from one examiner to another.[11] A cheaper, faster and easily accessible diagnostic method would greatly benefit patients with potential glomerular diseases.

24-hour urine collection is considered as the gold standard to asses proteinuria and albuminuria. The ratio of urinary protein to creatinine (uPCR) and albumin to creatinine (uACR) in a random urine sample is far more convenient than 24-hour collection and is considered as the preferred method to asses proteinuria and albuminuria. The urinary albumin-total protein ratio (uAPR) represents a potential easy to use method of differentiating the source of hematuria. At an uAPR cutoff value of 0.59 mg/mg, sensitivity was 97.3% and specificity was 100% in detecting glomerular disease,[11,12] showing potential to be used for early diagnosis. Although renal biopsy is the confirmatory step for the diagnosis of glomerular diseases,[13] alternative diagnostic tools can be developed. This study aimed to assess the performance of urinary albumin-total protein ratio as a diagnostic tool for the diagnosis of glomerular diseases in patients presenting with hematuria and compare it with percentage of dysmorphic RBC in urine.

METHODS

This cross-sectional comparative study was conducted in the Department of Nephrology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh from July 2018 to August 2019 between two groups of patients. One group of patients were selected from diagnosed cases of glomerular hematuria, confirmed by renal biopsy, done by spring loaded gun biopsy needle (16G). The renal tissue was sent for histopathological and direct immunofluorescence examination within 1 hour to the Department of Pathology, BSMMU. Another group of patients were selected from diagnosed cases of non-glomerular hematuria having isomorphic RBC on phase contrast microscopy with structural and pathological conditions visible on imaging.[14,15] Renal biopsy was not done on non-glomerular hematuria patients for ethical concerns. Purposive sampling technique was used. Ninety-six patients were enrolled in the study, 48 in each group. Pregnant women were excluded from the study along with patients suffering from bleeding disorder and on anti-coagulation drugs. All patients' fresh urine samples were collected for urine R/M/E.

After diagnosing hematuria, all patients were asked to provide 10 ml of urine for phase contrast microscopy to determine %dRBC. The MULTIGENT Microalbumin assay was used to measure urinary microalbumin. The ARCHITECT-C system was used to measure urinary protein levels and urinary creatinine levels. Hematuria was defined as ≥3 RBCs/HPF in spun urine.[2] Glomerular hematuria was defined as hematuria arising from renal parenchyma confirmed by renal biopsy.[14,16,17] Renal

biopsy was done on patients presenting with hematuria and uPCR > 0.5g protein/g creatinine.[18] Urinary albumin-total protein ratio (uAPR) was calculated by dividing spot urinary albumin by spot urinary total protein, expressed as mg/mg.[12]

Data were collected using a semi-structured interviewer assisted questionnaire and by recording laboratory and histological findings. All data were recorded systematically in pre-tested data collection forms. Quantitative data were expressed after analysis as mean with standard deviation. Categorical data were expressed as frequency and percentage. Statistical analyses were performed by using Windows® based computer software with Statistical Packages for Social Sciences (SPSS-25) (SPSS Inc, Chicago, IL, USA). Comparison of differences between continuous variables were done by unpaired t-test between two groups. For all statistical test, p-values ≤0.05 was considered significant.

RESULTS

Among the diagnosed cases of glomerular and non-glomerular hematuria, 60.4% and 68.7% samples were from 18 to 40 years age group with mean age of 35.52 years and 38.38 years respectively (Table I). In both group of patients, study population were predominantly male, 70.8% among patients with glomerular hematuria and 58.3% among patients with non-glomerular hematuria.

Table I Descriptive statistics of the study population

Criteria		Diagnosed cases of glomerular hematuria (n = 48)	Diagnosed cases of non-glomerular hematuria (n = 48)
Age in years			
	≤30	16 (33.3)	16 (33.3)
	31 – 40	13 (27.1)	17 (35.4)
	41 – 50	17 (35.4)	9 (18.8)
	> 50	2 (4.2)	6 (12.5)
	Mean ± SD	35.52 ± 11.42	38.38 ± 10.62
Sex			
	Male	34 (70.8)	28 (58.3)
	Female	14 (29.2)	20 (41.7)

Data are presented as n (%) or mean ± SD.

%dRBC was found to be 25.63% and 15.17% for patients with glomerular and non-glomerular hematuria respectively whereas uAPR was found to be 0.65 mg/mg and 0.40 mg/mg respectively (Table II). Both %dRBC and uAPR, showed statistically significance.

Table II Distribution of study population according to study parameters

Criteria	Diagnosed cases of glomerular hematuria (n = 48)	Diagnosed cases of non-glomerular hematuria (n = 48)	P Value
Percentage of dysmorphic RBC (%dRBC)	25.63 ± 14.86	15.17 ± 6.86	<0.001 [a]
Urinary albumin-total protein ratio (uAPR)	0.65 ± 0.08	0.40 ± 0.10	<0.001 [a]

[a] = Unpaired t-test was done to measure the level of significance and data were expressed as mean ± SD

Receiver Operating Characteristic Curve (ROC) was used to identify the best fitting value for both uAPR and %dRBC for differentiating patients with glomerular hematuria from patients with non-glomerular hematuria (Figure 1).

Youden's Index was used to determine the best fitting cutoff value for uAPR, the cutoff value was 0.57 mg/mg and for %dRBC the cutoff value was 22.5% (Table III). Using these cutoff values, sensitivity and specificity of uAPR was found to be 81.8% and 95.5% respectively in differentiating glomerular from non-glomerular hematuria (Table IV). For %dRBC, sensitivity and specificity were found to be 54.5% and 86.4% respectively (Table V).

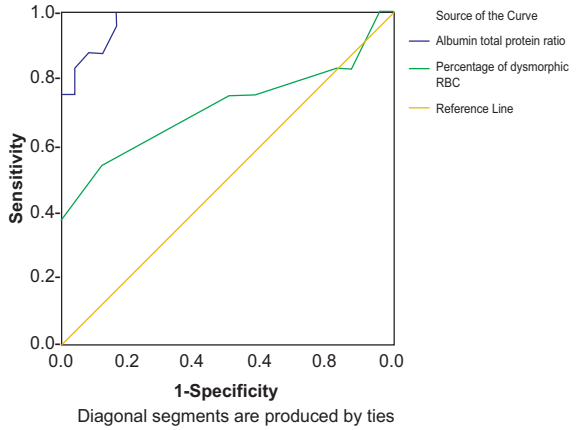

Figure 1 Receiver Operating Characteristic Curve (ROC)

Table III Statistics from ROC curve

Study parameters	Area under the curve	95% CI	Cutoff value according to Youden's Index
uAPR	0.98	0.95 - 0.98	0.57
%dRBC	0.71	0.54 – 0.82	22.5

Table IV Youden-Index to find out best cut-off value of uAPR for differentiating glomerular and non-glomerular hematuria

uAPR (mg/mg)	Sensitivity	1 – Specificity	Specificity	Youden index
.4100	1.000	.455	0.545	0.545
.4450	1.000	.364	0.636	0.636
.4650	1.000	.273	0.727	0.727
.4850	1.000	.227	0.773	0.773
.5000	1.000	.182	0.818	0.818
.5150	.955	.182	0.818	0.773
.5250	.864	.136	0.864	0.727
.5450	.864	.091	0.909	0.773
.5700	**.818**	.045	**0.955**	0.773
.5850	.773	.045	0.955	0.727
.5950	.727	.045	0.955	0.682

Table V Youden-Index to find out best cut-off value of %dRBC for differentiating glomerular and non-glomerular hematuria

%dRBC	Sensitivity	1 – Specificity	Specificity	Youden index
1.00	1.000	1.000	0.000	0.000
3.50	1.000	.955	0.045	0.045
6.00	.818	.864	0.136	-0.045
8.50	.818	.818	0.182	0.000
12.50	.727	.591	0.409	0.136
17.50	.727	.500	0.500	0.227
22.50	.545	.136	0.864	0.409
27.50	.364	0.000	1.000	0.364
35.00	.273	0.000	1.000	0.273
45.00	.136	0.000	1.000	0.136
51.00	0.000	0.000	1.000	0.000

DISCUSSION

For patients with hematuria, without thorough and extensive investigations, it is very difficult to identify the site of the hemorrhage. This often leads to delayed diagnosis. PCM to determine the %dRBC in urine has been in practice as an easy to use, cost saving and noninvasive method for differentiating glomerular from non-glomerular hematuria.[5,19] However %dRBC is modified by pH, osmolality or routine preparation procedure whereas uAPR shows no such change and has been suggested as an important diagnostic tool for glomerular hematuria.[11,12] Our study tried to determine if the uAPR could be used as a routine rapid screening test for glomerular hematuria.

In our study, %dRBC for diagnosed cases of glomerular and non-glomerular hematuria was 25% and 15% respectively. This was found to be 25% for diagnosed cases of glomerulonephritis with hematuria and 12% for patients with non-glomerular diseases with hematuria in a 2010 study, which is consistent with our study findings.[10] In our study, mean uAPR for diagnosed cases of glomerular and non-glomerular hematuria were 0.65 mg/mg and 0.40 mg/mg respectively. In a study done by Noriko mean uAPR for diagnosed cases of glomerular and non-glomerular hematuria were 0.72 mg/mg and 0.35 mg/mg respectively. [11] In another study, mean uAPR were 0.73 mg/mg and 0.41 mg/mg for diagnosed cases of glomerular and non-glomerular hematuria respectively.[12] These are also consistent with current study findings.

ROC curve analysis of %dRBC showed that, the area under the curve (AUC) was 0.713 and with a cut-off value of 22.50%, the sensitivity was 54.5% and specificity was 86.4% to differentiate between glomerular and non-glomerular hematuria. In a 2010 study by Crop, they reported findings similar to present study where at a cut-off value of 20%, the sensitivity and specificity was 54% and 91% respectively.[10] Another 2019 study reported findings similar to present study where the sensitivity and specificity was 59.6% and 82.1% respectively at a cut-off value of >10% dRBC.[20] Our ROC curve analysis of uAPR showed that, the AUC was 0.975 and a cut-off value of 0.57 mg/mg, has got the sensitivity of 81.8% and specificity of 95.5% to differentiate between glomerular from non-glomerular hematuria. A previous study found that AUC to be 0.99 which is very close to the present study and at a cut-off value of 0.59 mg/mg.[12] The sensitivity and specificity of uAPR was 97.3% and 100% respectively in that study. In a 2005 study by Naka found the best cut-off value of 0.57, closer to the findings of present study.[21] The sensitivity and specificity were lower in present study. This may be due to diurnal variation of urinary protein excretion, differences in age group, gender, body mass index, ethnicity and small sample size.

Conclusion

A cut-off value of 0.57 mg/mg of uAPR has been found to differentiate glomerular and non-glomerular hematuria

with the higher level of sensitivity and specificity compared to %dRBC using 22.5% as a cut-off value. uAPR appears to be a simple, accessible and reliable investigation to identify glomerular hematuria and can be used as a routine investigation in case of an undiagnosed case of hematuria. Further studies with larger sample size from multiple centers may be conducted to relate to the findings of this study.

Limitations

This study was conducted with patients only from one center. Samples were collected once from each patient, which may not generate accurate and reliable results all the time. Purposive sampling technique may have increased the chance of selection bias.

Ethical issues

Before conducting the study, approval was taken from the Ethical Committee (Institutional Review Board, IRB) of BSMMU (BSMMU/2018/12541). Necessary precautions were taken to protect privacy, anonymity and confidentiality of the information given by the respondents. Participants enjoyed the right to withdraw themselves from the study at any time. An informed written consent was obtained from the respondents during enrollment.

Conflict of interest: Nothing to declare.

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