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Determining Glomerular and Non-Glomerular Hematuria Dysmorphic Red Blood Cell: Study on Automatic Urine Analyzer

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ABSTRACT

Hematuria is a sign of glomerular and non-glomerular kidney disease. Erythrocytes that pass through the glomerulus undergo a change of shape to become dysmorphic. Dysmorphic Red Blood Cells (dRBC) can be screened using a urine analyzer based on flow cytometry to distinguish between the glomerular and non-glomerular causes of hematuria. The purpose of this study was to determine the diagnostic performance of the flow cytometry-based dRBC urine analyzer to distinguish glomerular hematuria from non-glomerular hematuria. This study used a cross-sectional research design at the Clinical Pathology Installation of Dr. Moewardi Hospital in Surakarta. The subjects of the study were patients with hematuria at the Outpatient Clinic and Internal Medicine Ward, Nephrology sub-division, Pediatric Outpatient Clinic, nephrology sub-division, and Urology Surgery Outpatient Clinic from March to July 2021. The diagnostic test was carried out after the determination of the cut-off value of dRBC using the ROC curve and AUC value analysis. The results showed that the cut-off value of dRBC for glomerular and non-glomerular hematuria was 67% and had the best analytical performance with a sensitivity of 91%, specificity of 85%, AUC of 0.890 (95% CI: 0.832–0.947; p= <0.001). In this study, dRBC parameters can be used for screening and diagnosis of glomerular and non-glomerular hematuria. Further research needs to be carried out using other more specific methods such as the examination of urine sediment with a phase contrast microscope.

Keywords: Glomerular hematuria, non-glomerular hematuria, dysmorphic red blood cells

INTRODUCTION

Hematuria is an abnormal condition characterized by the presence of erythrocytes in the urine. Causes of hematuria can be glomerular or non-glomerular.[1] The pathophysiology of hematuria depends on the anatomic location of the urinary tract where the bleeding occurs. Erythrocytes originate from various locations in the urinary tract, from the glomerular basement membrane to the distal urethra. If the bleeding comes from the nephrons, it is called glomerular hematuria. The morphology of these erythrocytes can be normal or abnormal. Erythrocytes that pass through the renal tubules change shape to become dysmorphic. Dysmorphic Red Blood Cells (dRBC) are red blood cells with changed morphological shape, and variations in size and are accompanied by loss of hemoglobin. The presence of dRBC and/or erythrocyte casts in the urine indicates glomerular or non-glomerular hematuria.[2]

The dRBC parameter from an automatic urine analyzer is determined from flow cytometric analysis and is intended as an initial screening test for hematuria patients. Hematuria patients with cut-off dRBC with erythrocyte casts are glomerular hematuria and require further medical follow-up. Hematuria patients with dRBC < cut-off with erythrocyte casts might be a combination of glomerular and non-glomerular hematuria and are therefore referred to the Nephrology Department. Hematuria patients with dRBC < cut-off without erythrocyte casts are non-glomerular hematuria and should be referred to the Urology Department. Urinalysis using flow cytometry can distinguish the types of erythrocytes into 3 groups, such as dRBC, isomorphic and mixed erythrocytes.[3]

METHODS

The study was conducted from May to July 2021 using a cross-sectional design, with samples taken consecutively. Subjects consisted of all patients with hematuria who underwent an examination at the Internal Medicine Outpatient Clinic, Nephrology-Hypertension sub-section, Pediatric Outpatient Clinic, Nephrology sub-section, Urological Surgery Outpatient Clinic, and Inpatient Ward of Moewardi Hospital Surakarta (RSDM) from May to July 2021. The hematuria group was determined based on the American Urologic Association (AUA) criteria, which consists of

immunoglobulin (Ig)A nephropathy, Lupus Nephritis (LN), membranoproliferative glomerulonephritis (MPGN), focal segmental glomerulosclerosis (FSGS), Alport's syndrome, infection-related glomerulonephritis (IRGN), post-infectious glomerulonephritis (PIGN). The non-hematuria group consisted of acute cystitis, urinary stones, benign and malignant kidney tumors, polycystic kidney disease, sickle cell disease, pyelonephritis, ureteral stricture, renal trauma, NSAID therapy, cyclophosphamide therapy, anticoagulant therapy, coagulation disorders, and coagulopathy.[4]

Urinalysis was performed in the Clinical Pathology Laboratory, Moewardi Hospital, Surakarta. Urinalysis was performed using Sysmex UX-2000 analyzer. A blood chemical test was carried out using Cobas C-311 and Advia 1800 device.

Statistical analysis was performed using SPSS ver. 16.0. The difference between the two independent groups was analyzed using the Mann-Whitney U test, and the cut-off urinary dysmorphic red blood cell was determined using the ROC curve. The diagnostic sensitivity and specificity of urinary dysmorphic red blood cells were calculated using a 2x2 table. A p-value < 0.05 was considered as statistically significant, with a 95% Confidence Interval (CI).

The biomedical research Ethics Committee approved this study of Sebelas Maret University, Faculty of Medicine/RSDM in Surakarta, recommendation number 606/V/HREC/2021.

RESULTS AND DISCUSSIONS

There were 302 subjects in this study who were diagnosed with hematuria. The research subjects consisted of 134 (44%) male patients and 168 (55%) female patients, 56 (18%) glomerular hematuria patients and 246 (81%) non-glomerular hematuria patients. The glomerular hematuria group consisted of 56 patients with 12 (21%) male patients and 44 (79%) female patients with ages ranging from 17 (2-66) years. There were total of 246 patients with non-glomerular hematuria, which consisted of 122 (50%) male patients and 124 (50%) female patients with ages ranging from 58 (13-87) years (Table 1).

Table 1. The characteristics of research subjects

Parameter	Glomerular (N=56)	Non-Glomerular (N=246)	Total Subject (N=302)	p
Age (year)	17 (2-66)*	58 (13-87)*	54 (2-87)	**0.001**
Gender				
Male	12 (21%)	122 (49%)	134 (44%)	**0.001**
Female	44 (78%)	124 (50%)	168 (55%)	**0.001**
Serum urea (mg/dL)	33 (10-423)*	49.50 (2.1-430)*	43 (2.1-430)*	**0.013**
Serum creatinine (mg/dL)	0.65 (0.20-17.80)*	1.2 (0.3-124)*	1.0 (0.2-124)*	**0.001**
Serum albumin (mg/dL)	2.95 (1.10-4.90)*	3.3 (1.6-5.1)*	3.3 (1.1-5.1)*	**0.011**
Protein in urine (mg/dL)	300 (3.72-1000)*	30 (0-1000)*	30 (30-1000)*	**0.001**
Urine blood (/µL)	108,45 (22.80-12675,60)*	222,9 (22-45894,6)*	178,75 (22-45894,6)*	**0.001**
eGFR (mL/min)	87.87 (3.72-308,68)*	51.98 (0.54-264,76)*	55.03 (0.54-308,68)*	**0.001**

Note: * Data were not normally distributed (median (min-max)); Mann-Whitney U-test, significant at p < 0.05; mg/dL: milligrams/deciliter; µL: microliter; eGFR: estimated Glomerular Filtration Rate; mL/min: milliliter/minute, N: number of subjects

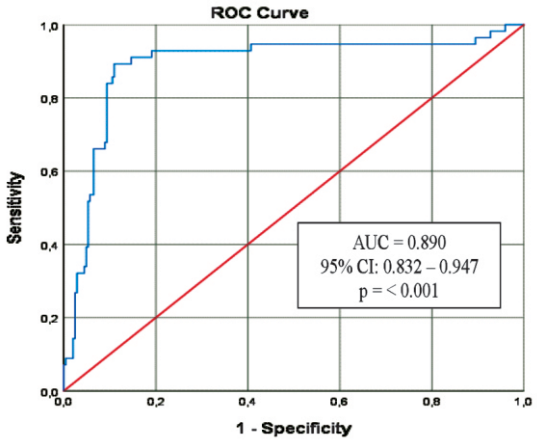

Figure 1. The cut-off ROC curve of dRBC based on the diagnosis of glomerular hematuria and non-glomerular hematuria

Based on the ROC curve, the cut-off value for dRBC was 67% with an AUC value of 0.890 (95% CI: 0.832–0.947; p=0.001) with a sensitivity of 91% and a specificity of 85%. The graph of the intersection of the sensitivity and specificity values is shown in Figure 1.

Patients were divided into 2 groups with a dRBC percentage of 67% and groups with a dRBC percentage of < 67%. The study subjects consisting of 302 patients were divided into a group of 87 patients with a dRBC percentage of 67% and a group of 215 patients with a dRBC percentage < 67%. A total of 87 patients with a dRBC percentage of 67% consisted of 51 patients with glomerular hematuria and 36 patients with non-glomerular hematuria. A total of 215 patients with dRBC percentage < 67% consisted of 5 patients with glomerular hematuria and 210 patients with non-glomerular hematuria. The dRBC value in the glomerular hematuria group was 88 (1.10-99.30)%, while the non-glomerular hematuria group was 10.55 (0.61-98.19)% with a p-value of 0.001 (Table 2).

Serum urea, serum creatinine, serum albumin, and urine blood levels were significantly different between the glomerular and non-glomerular hematuria groups (p-value <0.005). The results of the test also showed that the value of these parameters was higher in the non-glomerular hematuria group, indicating that these parameters played less role in the diagnosis of glomerular hematuria. This condition was in accordance with the theory suggesting that non-glomerular hematuria originates from abnormalities in the urinary tract, Urinary Tract Infections (UTIs), urinary stones, and malignancies in the kidneys and their ducts. These diseases are dominated by obstruction of the urinary tract, resulting in blocked excretion of metabolic products from the kidneys, leading to increased serum urea and serum creatinine levels.[5]

The lower serum albumin and higher proteinuria in glomerular hematuria (p-value < 0.005) in this study were consistent with the theory suggesting that albuminuria is an important indicator of glomerular damage and is more common in glomerular hematuria. Glomerular proteinuria, one classification of proteinuria, is caused by disruption, damage, or lesions in the glomerulus (nephrotic syndrome, LN), resulting in an increase in protein filtration through the damaged glomerular capillary wall. This loss of protein through urine also causes decreased serum albumin levels. According to Kim *et al.*, the combination of dRBC and proteinuria improves the detection of glomerular hematuria.[1]

Dysmorphic red blood cells between the subjects of glomerular and non-glomerular hematuria in this study had a significant difference (p<0.001), with higher dRBC in glomerular hematuria compared to non-glomerular hematuria (88(1.10-99.30)% vs. 10.55 (0.61-98.19)%), indicating that dRBC can be used for the diagnosis of glomerular hematuria. Based on the ROC curve, the cut-off value of dRBC for glomerular and non-glomerular hematuria was 67%, and the AUC value was 0.890 (95% CI: 0.832–0.947; p=0.001) with a sensitivity of 91% and a specificity of 85%. The results of sensitivity and specificity > 80% indicate that a dRBC examination can serve as a screening and diagnosis of glomerular hematuria. The dRBC biomarker can also be used to screen patients who need a kidney biopsy, supported by a study by Kim *et al.*, which concluded that urinalysis

Table 2. The dRBC flow cytometry cut-off diagnostic test results in 67% of the clinician's diagnosis

Parameter	Formula	Results
Sensitivity	$\frac{a}{a+c}$	91.07% 95% CI : 0.783-0.842; p=0.001
Specificity	$\frac{d}{d+b}$	85.36% 95% CI : 0.545-0.617; p=0.001
PPV	$\frac{a}{a+b}$	58.62%
NPV	$\frac{d}{c+d}$	97.67%
LR (+)	$\frac{Sn}{[1 - Sp]}$	6.22
LR (-)	$\frac{[1 - Sn]}{Sp}$	0.10
Accuracy	$\frac{a+b}{a+b+c+d}$	86.42%

Description: a: true positive, b: false positive, c: false negative, d: true negative, Sn: sensitivity, Sp: specificity, CI: Confidence Interval, PPV: Positive Predictive Value, NPV: Negative Predictive Value, LR(+): Likelihood Ratio (+), LR(-): Likelihood Ratio (-)

must be performed for hematuria patients with dRBC > 80%, erythrocyte cast > 1, acanthocytes > 5%, gross hematuria 2 of 3.[1]

The results of this study found dRBC 67% in 36 (41%) subjects but included in the criteria for non-glomerular hematuria (false positive). The condition that might cause the false positive results was the coincidence between UTI and other diseases such as Diabetes Mellitus (DM) and hypertension, which has started to develop into nephropathy, leading to UTI indication but with significant dRBC and proteinuria in the urinalysis results.[6] The research data showed that there were 31 patients with DM, 15 hypertensive patients, and 20 UTI patients, which might undergo DM or hypertension nephropathy especially 15 patients with eGFR values < 45 mL/min. Therefore, clinicians need to consider the laboratory parameter for diabetic nephropathy and hypertension, such as urinary albumin to creatinine ratio. The study data also showed that three patients with leptospirosis had dRBC 67% and eGFR < 45 mL/min. According to Gompf, leptospira bacteria migrate to the interstitial tissue of the tubules and tubular lumen and then cause interstitial nephritis and tubular necrosis. Renal impairment in leptospirosis is included in the criteria for non-glomerular hematuria, it is possible that this patient already has kidney failure, resulting in glomerular damage.[7] There was also one patient with autoimmune hemolytic anemia (AIHA) with dRBC 95% and eGFR 116 mL/min. Intravascular hemolysis can cause tubular necrosis. The heme in nephron cells causes nephrotoxicity due to renal hypoperfusion, direct cytotoxic effects, and tubular occlusion of cylinders. Patients with AIHA meet the criteria for non-glomerular hematuria, leading to the possibility that these patients also have glomerular damage due to direct cytotoxic effects.[7]

The results of this study also found 5 (2.32%) people with dRBC < 67% but were classified as glomerular hematuria (false negative). Conditions that often underlie false negative results are mild to moderate Acute Kidney Injury (AKI), grade 3-4 CKD, and response to steroid therapy in LN. In this condition, the degree of glomerular damage is moderate, and the glomerular damage is still reversible or in remission.[8] The research data showed that there were 3 LN patients and 2 CKD patients with dRBC < 67%. This was presumed as an LN that went into remission after therapy; therefore, dRBC can be used as one of the parameters for evaluating therapy in LN. Research by Dong *et al*. and Martinez *et al*. showed that kidney biopsy in LN had a degree of similarity with the results of dRBC percentage in both

Table 3. Differences in the cut-off value of dRBC flow cytometry with other researchers

Researcher	Year of Publication	Number of Samples	Population	Gold Standard	Cut-off dRBC	Sn	Sp
Dong *et al.*[6]	2016	198	DM, hypertension, NS	Phase contrast microscope	80%	25%	97%
Koo *et al.*[9]	2016	411	CKD, DM, LN, IgA nephropathy, kidney stones, bladder tumors, BPH, UTI	Phase contrast microscope	40%	63.7%	72.7%
Anju[5]	2018	800	UTI, kidney stones, SLE, NS, DM, hypertension	Phase contrast microscope	25%	94.6%	36.9%
Hamadah *et al.*[10]	2018	482	SLE, LN, IgA nephropathy, thrombosis, hypertension, tubulointerstitial, DM	Phase contrast microscope	25%	20.4%	96.3%
Kim *et al.*[1]	2019	103	SLE, LN, IgA nephropathy, kidney stones, bladder tumors	Phase contrast microscope	40.5%	70.20%	76.80%

Description: dRBC: Dysmorphic Red Blood Cell; Sn: sensitivity; Sp: specificity; UTI: Urinary Tract Infection; SLE: Systemic Lupus Erythematosus; NS: Nephrotic Syndrome; DM: Diabetes Mellitus; CKD: Chronic Kidney Disease; IgA: immunoglobulin A; BPH: Benign Prostatic Hypertrophy

pre-and post-therapy. This dRBC parameter will be more meaningful when combined with other parameters such as urine protein.[6]

The cut-off value of dRBC according to Anju varies between 1-50%, and the main population used in this study was patients with UTIs and glomerulonephritis.[5] Research by Jiang *et al*. using the Sysmex UF-1000i urinalysis device (flow cytometry method) found that the dRBC cut-off value was 80% with the main population being subjects with UTIs, urinary stones, and LN.[1] In a population of patients with type 2 diabetes, hypertension, and nephrotic syndrome with hematuria obtained a dRBC cut-off value of 80% with a specificity of 93% and a PPV of 85%, almost the same as the results of this study. The results of the cut-off obtained by the authors can mainly be applied to patients in a population with DM, hypertension, UTIs, and nephrotic syndrome.[6]

A previous study by Kim *et al.* on dRBC examination to differentiate glomerular and non-glomerular hematuria obtained a cut-off of 41%, AUC value of 0.745, sensitivity of 70%, and specificity of 77% using the Sysmex UF- 1000i.[1] A similar study conducted by Anju, using the Sysmex UX-2000 device obtained a dRBC cut-off of 25%, the sensitivity of 95%, and specificity of 36.9% (Table 3). The results of the study using a gold standard phase contrast microscope obtained a dRBC cut-off of 25%, sensitivity of 90%, and specificity of 100%. The difference in the cut-off value between this study and other studies was probably due to differences in the use of the gold standard, this study used the gold standard for clinician diagnosis, while other researchers used urine sediment examination using a phase contrast microscope.[1]

No sediment examination using a phase contrast microscope (as the gold standard for identifying dRBC in urine) remains the limitation of this study.

CONCLUSIONS AND SUGGESTIONS

The dRBC cut-off value of 67% for glomerular and non-glomerular hematuria had the best diagnostic performance with a sensitivity of 91%, specificity of 85%, and AUC of 0.890 (95% CI: 0.832–0.947; p=<0.001); therefore, it can be used for screening and diagnosis of patients with glomerular and non-glomerular hematuria.

Further studies were needed to compare the dRBC cut-off value from the automatic urine analyzer with another more specific gold standard, such as a microscopic examination of urine sediment with a phase contrast microscope.

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