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Dysmorphic erythrocytes are superior to hematuria for indicating non-diabetic renal disease in type 2 diabetics

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ABSTRACT

Aims/Introduction: There are sparse and limited studies on erythrocyte morphology in renal biopsy identifying nephropathic patients among type 2 diabetics. The present study sought to clarify the predictive value of dysmorphic erythrocytes in type 2 diabetics with non-diabetic renal disease and influences on hematuria.

Materials and Methods: We examined 198 patients with type 2 diabetes who underwent kidney biopsies between 2012 and 2013. Hematuria was defined as >3 or >10 red blood cells per high-power field (RBCs/hpf) in urine sediment. If >80% of the erythrocytes were dysmorphic, glomerular hematuria was diagnosed. Clinical findings and predictive value of dysmorphic erythrocytes were compared between patients with hematuria (n = 19) and those without (n = 61). The potential risk factors for hematuria among diabetic nephropathy patients were also screened.

Results: There was a statistically significant difference between the diabetic nephropathy group and the non-diabetic renal disease group (6.6 vs 16.8%; P = 0.04) when the demarcation point of hematuria was 10 RBCs/hpf. When the definition of hematuria was based on an examination of urinary erythrocyte morphology, a marked difference was seen (3.3 vs 24.8%; P < 0.001). Glomerular hematuria showed high specificity and a positive predictive value (0.97 and 0.94, respectively) in non-diabetic renal disease. A multivariate analysis showed that nephrotic syndrome was significantly associated with hematuria (odds ratio 3.636; P = 0.034).

Conclusions: Dysmorphic erythrocytes were superior to hematuria for indicating non-diabetic renal disease in type 2 diabetics. Nephrotic syndrome was an independent risk factor for hematuria.

INTRODUCTION

It is commonly accepted that microscopic hematuria is an uncommon symptom in diabetic nephropathy (DN), which suggests the presence of non-diabetic renal disease (NDRD). American Diabetes Association guidelines consider hematuria an indication for renal biopsy in patients with diabetes mellitus[1]. There are two types of erythrocytes in urine sediment: isomorphic (indicating non-glomerular hematuria) and dysmorphic (indicating glomerular hematuria). Only glomerular hematuria represents kidney disease. Microscopic hematuria in DN patients is glomerular hematuria[2,3]. The most likely mechanism could involve pathological changes in the glomerular basement membrane and ruptured pseudoaneurysms[4]. Urinary erythrocyte morphology examined by phase-contrast microscopy is a 'classical' and important diagnostic tool, because it helps distinguish the causes of hematuria[5]. However, screening for urinary dysmorphic erythrocytes in type 2 diabetics with microscopic hematuria has become an overlooked technique. In

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recent studies, the reported prevalence of microscopic hematuria in patients with biopsy-confirmed isolated DN even reached 32.3–78%[6–8]. Furthermore, given the definition of hematuria and subjects with diabetes among the patients studied, non-glomerular hematuria might interfere significantly. Thus, we hypothesized that a failure to identify the site of bleeding leads to a high reported prevalence of microscopic hematuria in diabetic patients.

A finding of acanthocyturia is indicative of NDRD in diabetic patients with proteinuria, but NDRD is indicated without a pathological diagnosis[9]. Few studies have discussed the relationship between microscopic hematuria and DN in type 2 diabetics, but without any examination of urinary erythrocyte morphology[10,11]. We studied the prevalence of microscopic hematuria and dysmorphic erythrocytes in patients with pathologically diagnosed DN and NDRD to analyze whether dysmorphic erythrocytes occur in both types of renal lesions or whether they are specific to NDRD. In type 2 diabetics, a finding of >80% dysmorphic erythrocytes in urine sediment could point to a non-diabetic, potentially treatable glomerulopathy for which a renal biopsy might be indicated[9].

MATERIALS AND METHODS

Study Population

In total, 221 consecutively diagnosed type 2 diabetics who underwent a renal biopsy at the Chinese People's Liberation Army General Hospital (Beijing, China) between January of 2012 and December of 2013 were considered for the study. The diagnosis of type 2 diabetes was made by experienced endocrinologists. All patients including those with suspected DN and NDRD with persistent overt proteinuria and nephropathy, as diagnosed by renal biopsy, were admitted to our hospital. All patients provided written informed consent for the renal biopsy. The exclusion criteria were a pathological diagnosis of DN combined with NDRD (n = 9), cases in which the primary disease presented with a microscope field full of red blood cells (RBCs) and white blood cells (e.g., systemic lupus erythematosus [n = 5]), and patients with continuous pyuria (n = 2) or anuria (n = 2). Those with urolithiasis and those without erythrocyte morphology data or uncertain results were also excluded (n = 5). Patients with hematological disease, such as sickle cell disease, were also excluded (n = 0). Thus, of the 221 patients, 198 were finally enrolled. The study received ethics approval from the Medicine Ethics Committee of Chinese PLA General Hospital (Approval No. S2014-012-01).

Of the 198 patients, 128 were men (64.65%). The mean age at renal biopsy was 49.98 ± 10.42 years. The mean known duration of type 2 diabetes was 72.78 ± 86.82 months, and the mean serum creatinine (Scr) level was 123.50 ± 94.46 μmol/L. The mean estimated glomerular filtration rate was 79.67 ± 42.07 mL/min/1.73 m^2 using the Chronic Kidney Disease Epidemiology Collaboration equation[12]. The mean hemoglobin level was 127.67 ± 22.15 g/L, the glycated hemoglobin (HbA1c) level was 6.86 ± 1.43%, and the 24-h urinary total protein level was 3.39 ± 2.98 g. The HbA1c level was measured by high-performance liquid chromatography (normal range 4–6%).

Patients with DN had longer durations of type 2 diabetes, and their renal function was more severely impaired than patients with NDRD (Table 1).

Urinalysis

Before renal biopsy, the first morning specimen of midstream urine was collected. Urinalysis was carried out within 2 h of micturition. Whenever possible, three urine samples collected on three separate days before the renal biopsy were analyzed to increase the sensitivity[13]. The number of samples ranged between one and three, depending on the waiting period for the renal biopsy. There were some patients with less than three urine samples whose urine RBCs increased by less than twofold vs prebiopsy. Their results of erythrocyte morphology after biopsy after more than 1 week were included. The urine samples had to be collected before treatment of the renal pathology.

Urinary erythrocyte morphology was examined by a single specially trained professional technologist who was experienced (i.e., who carried out more than 600 urine sediment examinations per month). First, 10 mL of urine were centrifuged (377.33 *g*, 10 min). Then, 9.5 mL of the supernatant were discarded. The sediment was resuspended in 0.5 mL of urine. Next, the suspension (20 μL) was investigated in a Fuchs–Rosenthal counting chamber by phase-contrast microscopy. If a patient had provided only one urine sample, the results of a routine urine test from the hospital's clinical laboratory were used. Hematuria was defined as >3 RBCs per high-power field (hpf) in at least two urine samples[14,15]. Quantitative threshold values of RBCs have been used as a basis for diagnosis. If >80% dysmorphic erythrocytes were seen, glomerular hematuria was diagnosed[16–19].

Table 1 | Clinical and laboratory indexes of patients with diabetic nephropathy and patients with non-diabetic renal disease

	DN group	NDRD group	*P*-value
n	61	137	
Sex, male (%)	45 (73.8%)	83 (60.6%)	0.07
Age (years)	49.90 ± 9.24	50.03 ± 10.94	0.94
Diabetes duration (months)	144 (61.50–192)	6 (1–24)	<0.001
Hemoglobin (g/L)	117.56 ± 21.44	134.59 ± 20.43	<0.001
HbA1c (%)	7.18 ± 1.69	6.81 ± 1.28	0.10
Scr (μmol/L)	151.21 ± 85.49	111.16 ± 95.94	0.01
eGFR (mL/min/1.73 m^2)	58.33 ± 32.07	89.18 ± 42.60	<0.001
24-h urinary total protein (g)	3.41 (1.66–5.38)	2.08 (0.73–4.67)	0.02

DN, diabetic nephropathy; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; NDRD, non-diabetic renal disease; Scr, serum creatinine.

If a patient had provided a single sample, we used the single morphology result. If a patient had two samples, they were used if the two results were consistent, or excluded as an uncertain result. If a patient had three samples, we used two or three consistent results.

Renal Biopsy and Pathological Examination

All patients stopped taking agents with antiplatelet/anticoagulation activity 3 days before the renal biopsy. Patients with a high risk of thrombosis were allowed to restart anticoagulant and/or antiplatelet therapy at least 3 days after the renal biopsy, whereas the remaining individuals restarted treatment at least 0.5–1 months after the renal biopsy. Renal biopsies were carried out by two experienced nephrologists. No patients exhibited gross hematuria after the operations. The diagnosis of DN or NDRD was made by a single pathologist.

Statistical Analysis

Continuous variables are reported as means ± standard deviations and percentages; categorical data are reported as medians and 25–75th percentiles. The independent *t*-test was used to compare normally distributed continuous variables. Between-group differences in data for variables not normally distributed were analyzed using the Mann–Whitney *U*-test. The χ^2-test was used to compare categorical variables. Clinical parameters that were significant at the 0.05 level in a univariate logistic regression analysis were assessed to evaluate their contributions to hematuria. A *P*-value <0.05 was considered to indicate statistical significance. SPSS software (version 17; SPSS Inc., Chicago, IL, USA) was used for all analyses.

RESULTS

Performance Measures of Hematuria

Among the enrolled 198 patients, 80 (40.4%) had microscopic hematuria. The percentages of hematuria in the DN and NDRD groups were 31.3% and 43.8%, respectively, with no statistically significant difference ($P = 0.77$). However, there was a statistically significant difference between the groups (6.6% vs 16.8%, $P = 0.04$) when the demarcation point of hematuria was 10 (not 3) RBCs/hpf. When the definition of hematuria was based on the urinary erythrocyte morphological examination, a marked difference was evident (3.3% vs 24.8%, $P < 0.001$; Table 2).

We used three different definitions of hematuria: >3 RBCs/hpf, >10 RBCs/hpf and dysmorphic erythrocytes >80% in urine sediment (glomerular hematuria). These criteria were used to diagnose NDRD. For glomerular hematuria, the specificity and positive predictive values were high (0.97 and 0.94, respectively). If a patient had glomerular hematuria, the probability of NDRD was 0.97. Conversely, the rate of exclusion of NDRD was 0.94. Furthermore, glomerular hematuria had the maximum area under the receiver operator characteristic curve (0.61 vs 0.57 and 0.56; Table 3).

In total, 61 patients (30.8%) were diagnosed with DN among the 198 participants. We compared the clinical parameters between the DN patients with hematuria (group 1, $n = 19$) and those without hematuria (group 2, $n = 42$). Comparisons of the clinical characteristics and pertinent laboratory findings between the two groups are shown in Table 4. Among groups 1 and 2, nephrotic syndrome (NS) was found in 12 (63.2%) and 11 (26.2%) patients, respectively ($P = 0.006$). Urinary protein excretion was higher in group 1, but there was no statistically significant difference. There was no difference between the groups in terms of age, known duration of diabetes, hypertension, diabetic retinopathy (DR), HbA1c or Scr.

Table 2 | Comparison of the incidence of hematuria in the diabetic nephropathy and non-diabetic renal disease groups

Definition of hematuria (RBCs/hpf)	DN group, presence (%)	NDRD group, presence (%)	*P*-value
>2	24 (39.3%)	64 (46.7%)	0.29
>3	19 (31.1%)	60 (43.8%)	0.77
>5	15 (24.6%)	42 (30.7%)	0.33
>7	10 (16.4%)	31 (22.6%)	0.27
>8	7 (11.5%)	27 (19.7%)	0.13
>10	4 (6.6%)	23 (16.8%)	0.04
>15	3 (4.9%)	15 (10.9%)	0.17
Glomerular hematuria	2 (3.3%)	34 (24.8%)	<0.001

DN, diabetic nephropathy; NDRD, non-diabetic renal disease; RBCs/hpf, red blood cells per high-power field.

Table 3 | Predictive value of three different diagnostic criteria for hematuria

	>3 RBCs/hpf	>10 RBCs/hpf	Glomerular hematuria
Sensitivity	0.44	0.17	0.25
Specificity	0.69	0.93	0.97
Positive predictive value	0.76	0.85	0.94
Negative predictive value	0.35	0.33	0.36
ROC AUC	0.57	0.56	0.61

AUC, area under the curve; RBCs/hpf, red blood cells per high-power field; ROC, receiver operator characteristic curve.

Relationship Between DN and Hematuria

The results from the univariate logistic regression analysis showed that NS, D-dimer and brain natriuretic peptide were related to DN. However, NS showed collinearity with D-dimer and brain natriuretic peptide. Furthermore, DR was more meaningful than those two variables. Ultimately, in the multivariate predictive logistic regression analysis model, we used the variables NS and DR (Table 5).

Table 4 | Clinical characteristics and pertinent laboratory findings in diabetic nephropathy patients with and without microscopic hematuria

	Group 1 Hematuria (+) (n = 19)	Group 2 Hematuria (–) (n = 42)	P-value
Age (years)	51.32 ± 12.12	49.26 ± 7.70	0.43
Duration of diabetes (months)	132.84 ± 91.76	135.55 ± 90.58	0.92
NS, yes (%)	12 (63.2%)	11 (26.2%)	0.006
Hypertension, yes (%)	18 (94.7%)	38 (90.5%)	0.57
DR, yes (%)	13 (68.4%)	30 (71.48%)	0.97
Hemoglobin (g/L)	113.05 ± 20.27	119.59 ± 21.87	0.27
HbA1c (%)	6.77 ± 1.4	7.04 ± 1.44	0.21
BUN (mmol/L)	9.42 ± 3.28	9.32 ± 4.30	0.93
Scr (μmol/L)	132.74 ± 74.04	159.57 ± 89.77	0.26
eGFR (mL/min/1.73 m^2)	61.95 ± 26.69	56.70 ± 34.40	0.56
Urine protein excretion (g/24 h)	4.56 ± 2.75	3.47 ± 2.37	0.12

BUN, blood urea nitrogen; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; NS, nephrotic syndrome; Scr, serum creatinine.

Patient Profiles at Renal Biopsy

In total, 137 patients (69.2%) were diagnosed with NDRD. Membranous glomerulonephritis (46 patients, 33.6%) was the most common glomerular NDRD. A total of 30 (28.5%) patients were diagnosed with immunoglobulin A nephropathy, eight (5.8%) with obesity-related glomerulopathy and another 6 (4.4%) with minimal change disease. Glomerular hematuria was a frequent finding in immunoglobulin A nephropathy (18/39, 46.2%) and membranous glomerulonephritis (11/46, 23.9%), but rare in minimal change disease (1/6, 16.7%). There was no glomerular hematuria in obesity-related glomerulopathy.

DISCUSSION

There is a common consensus not to carry out renal biopsies in clinically diagnosed DN patients, but to do so in NDRD patients[20]. Thus, it is important to define the clinical characteristics and laboratory features to correctly indicate the presence of NDRD and to selectively carry out renal biopsies in those patients. Studies based on renal biopsies in diabetic patients have shown that the incidence of NDRD in patients with type 2 diabetes mellitus was much higher than in patients with type 1 diabetes mellitus[14,21]. The incidence of NDRD in the present study was 69.2%. The major pathological types were membranous nephropathy and immunoglobulin A nephropathy. Most NDRD patients require aggressive immunosuppressive treatment to obtain more favorable outcomes. Thus, making an accurate diagnosis of NDRD by non-invasive methods is more important for type 2 diabetic patients.

Table 5 | Univariate and multivariate logistic regression models of clinical findings associated with hematuria

	Odds ratio	95% Confidence interval	P-value
Univariate logistic regression			
NS	4.831	1.517–15.387	0.008
D-dimer	7.852	1.78–34.408	0.006
BNP	1.001	1.000–1.001	0.046
DR	0.975	0.254–3.745	0.971
Multivariate logistic regression			
NS	3.636	1.105–11.969	0.034

BNP, brain natriuretic peptide; DR, diabetic retinopathy; NS, nephrotic syndrome.

Some guidelines[1,22] and reports[23] have proposed that hematuria suggests non-diabetic glomerulopathy in diabetic patients. However, hematuria is a rather frequent finding in diabetic patients with renal injury. Indeed, several reports have suggested that hematuria is a sign of DN[2,24]. The incidence of hematuria was 32.3–78% in renal biopsy studies of patients with type 2 diabetes mellitus and proteinuria[6–8]. More importantly, hematuria had low specificity for the diagnosis of NDRD[25]. The incidence of hematuria was 0.69 in our data, consistent with other reports, but was not increased in NDRD patients when compared with isolated DN patients. Thus, the presence of hematuria does not generally indicate NDRD. The criteria and patterns of hematuria in diabetic patients must be studied further to help detect non-DN before a renal biopsy.

The definition of microscopic hematuria has not been uniform. In previous reports, there have been inconsistent criteria for hematuria that are pathologically significant; this warrants further investigation. According to current guidelines, the presence of >3 RBCs/hpf is considered clinically significant microscopic hematuria[26]. There have been various criteria, for both scientific research and the clinical diagnoses of hematuria: >2 RBCs/hpf[6], >3 RBCs/hpf[14,15], >5 RBCs/hpf[27], >10 RBCs/hpf[3], and >15 RBCs/hpf. As a result, the incidence of hematuria varied from 4.9 to 39.3%. The incidence of hematuria did not differ between the DN and NDRD groups when defined as >3 RBCs/hpf, but it did differ when defined as >10 RBCs/hpf. However, the latter definition had lower diagnostic efficiency. Thus, we studied the urinary erythrocyte morphology.

Scattered and limited studies have investigated urinary erythrocyte morphology in type 2 diabetics diagnosed with DN or NDRD by renal histopathology. We found that the incidence of glomerular hematuria was 3.3% in renal biopsy-proven DN patients; indicative performance was better in DN patients than in NDRD patients. A previous study observed that glomerular hematuria (hematuria comprising >5% acanthocytes of all red cells excreted) was seen in just 4% of all patients with clinically diagnosed DN[9]. In contrast, among all patients with NDRD, glomerular hematuria was found in 40%[9]. Because of the usual absence of renal biopsies in clinically diagnosed DN patients, some NDRD patients might be misdiagnosed with DN, thereby losing the opportunity for renal biopsy. Therefore, the prevalence of hematuria in DN

might have been overestimated. Nevertheless, microscopic inspection of urine sediment should be part of the non-invasive diagnostic work-up of diabetic patients with proteinuria to identify diabetic patients with hematuria who are likely to have NDRD[9]. Additionally, Kincaid-Smith *et al.*[28] reported that urine microscopy was often second only to renal biopsy in making a diagnosis. Other guidelines also stress the role of urinary sediment as a discriminating diagnostic instrument in patients with hematuria[29–32].

However, the problem is both a lack of professional and technical personnel to detect urinary erythrocyte morphology, and the standardized definition of dysmorphic erythrocytes. Simply, with the appearance of more effective methods, the examination of urinary erythrocyte morphology has become overlooked. Renal biopsy is an invasive test that is currently underused in type 2 diabetic patients, who require supplementary methods. This technique, the fastest and cheapest of all investigations, can provide a wealth of information for making a diagnosis. When examining increasing numbers of urine samples, the incidence of acanthocyturia increased in patients with NDRD and in patients with DN[9]. On analysis of three urine samples, the prevalence of acanthocyturia increased, improving the diagnostic accuracy. Another study confirmed that patients undergoing a renal biopsy had equivalent percentages of dysmorphic RBCs, both pre- and post-biopsy[33]. Therefore, the analysis of a patient's dysmorphic erythrocytes after a biopsy should be carried out to increase the diagnostic accuracy. The examination of urinary erythrocyte morphology should be carried out by an experienced technologist; it is a useful diagnostic tool, but only if strict criteria established in each laboratory are adhered to[33].

To date, the relationship of hematuria with clinical and laboratory variables in DN patients with type 2 diabetes has been described in only a few studies, and the details remain unclear. One study recruited patients with type 2 diabetes and biopsy-proven DN[3]. When compared with the non-hematuria group, the hematuria group had a longer known duration of diabetes mellitus, with a mean time of 108 months; a higher Scr level, with a mean value of 123.76 μmol/L; and a lower level of Scr, with a mean value of 45.2 mL/min[3]. Significant increases in the prevalence of NS (72%) and DR (57%) were also found in cases with hematuria, but not in those without hematuria[3]. Akimoto *et al.*[3] suggested that hematuria might be a common feature in patients with late-stage glomerular damage caused by diabetes. Conversely, our data and those from another study show no difference in these variables between groups[11]. A multivariate logistic regression analysis identified the presence of NS[3], the duration of diabetes[3] and the index of arteriolar hyalinosis[11] to be significant predictors of hematuria with DN. We found only the presence of NS to be higher in hematuria patients, and NS was the only independent predictor of hematuria in biopsy-proven DN patients with type 2 diabetes in the present study. The discrepancies between these different studies are partly the result of differences in the populations of diabetic patients examined.

Although the present study provides new information on the diagnostic value of dysmorphic erythrocytes in patients with type 2 diabetic nephropathy, it also has several limitations. First, the number of DN patients who had hematuria included in the present study was small, which likely means that the results might be underpowered to detect NS as a predictor of hematuria. However, these clinical observations drew our attention to a latent relationship between NS and hematuria. Further analysis involving a larger number of type 2 diabetic patients with pathologically defined DN from multiple centers is required. Second, the lack of a quantitative evaluation of morphological analyses of the kidney might cause performance degradation in a multivariate logistic regression analysis. However, in the absence of this information, the findings of the present study could still serve as a reference. Third, we used >80% dysmorphic erythrocytes as a criterion for glomerular hematuria, the specificity of which was lower than acanthocyturia, while the sensitivity was higher. The examination of three early morning urine samples taken on three different days before a renal biopsy should be recommended, because it increases the specificity of the method. Fourth, we analyzed only the efficacy of a hematuria-based diagnostic strategy in this retrospective study of patients who already had a diagnosis of DN or NDRD. A prospective study should be carried out to verify the validity of dysmorphic erythrocytes in the diagnosis of unknown individuals.

In summary, glomerular hematuria is rare in DN patients with type 2 diabetes mellitus. A renal biopsy should be considered when a type 2 diabetic patient with proteinuria shows >80% dysmorphic erythrocytes in a urine sample. We suggest that a urinary erythrocyte morphological examination should be part of the diagnostic work-up in those patients to identify which patients are likely to have NDRD. NS was the only independent predictor of hematuria in type 2 diabetic patients with DN.

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DISCLOSURE

The authors declare no conflict of interest.

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