Acanthocytes in the Urine

Useful tool to differentiate diabetic nephropathy from glomerulonephritis?

OBJECTIVE — The presence of hematuria has been suggested to indicate nondiabetic nephropathy in diabetic patients with proteinuria. However, hematuria is frequently found in patients with biopsy-proven diabetic glomerulosclerosis without nondiabetic nephropathy. Urine microscopy allows discrimination of glomerular hematuria, which is defined as acanthocyturia (urinary excretion of acanthocytes, which are dysmorphic erythrocytes with vesicle-like protrusions), from nonglomerular hematuria. We hypothesized that acanthocyturia is an uncommon finding in diabetic nephropathy, which suggests the presence of a nondiabetic nephropathy in diabetic patients with proteinuria.

RESEARCH DESIGN AND METHODS — Urine samples of patients with the clinical diagnosis of diabetic nephropathy (n = 68), of patients with biopsy-proven glomerulonephritis (n = 43), and of age-matched healthy control subjects (n = 20) were examined by phase-contrast microscopy for the presence of hematuria (≥8 erythrocytes/μl) and acanthocyturia. Acanthocyturia of ≥5% (5 acanthocytes among 100 excreted erythrocytes) was classified as glomerular hematuria; acanthocyturia of 2–4% was classified as suspected glomerular hematuria.

RESULTS — Hematuria was found in 62% of patients with the clinical diagnosis of diabetic nephropathy, in 84% of patients with glomerulonephritis, and in 20% of the healthy control subjects upon a single urine examination. In contrast, glomerular hematuria occurred in 4% of patients with diabetic nephropathy and in 40% of patients with glomerulonephritis (P < 0.001).

CONCLUSIONS — In contrast to hematuria, acanthocyturia is uncommon in patients with the clinical diagnosis of diabetic nephropathy. In diabetic patients with proteinuria, the finding of acanthocyturia points to nondiabetic glomerulopathies, and renal biopsy should be considered.

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The prevalence of microscopic hematuria in diabetic patients with macroalbuminuria may range between 12.5% (1) and 67–73% (2,3), depending on the population of diabetic patients studied and the definition of hematuria. The finding of microscopic hematuria in diabetic patients has been suggested to indicate nondiabetic glomerulopathy, which would demand further nephrological examination, including renal biopsy (1). However, renal biopsy is an invasive procedure with potentially severe complications, and careful histologic examination of renal tissue from diabetic patients with hematuria fairly often only demonstrates diabetic nephropathy without evidence of nondiabetic glomerulonephritis (isolated diabetic nephropathy) (1,4–7). In addition, it was recently reported that the prevalence of hematuria in diabetic patients with histologic evidence of nondiabetic nephropathy is only slightly (6) or not at all (8,9) increased when compared with patients with biopsy-proven isolated diabetic nephropathy. Therefore, it would be helpful to define a pattern of hematuria that characterizes a subgroup of patients who are most likely to have a nondiabetic glomerulopathy and to selectively perform renal biopsy in this subgroup of patients.

Urine erythrocyte morphology examined by phase-contrast microscopy allows differentiation of glomerular and nonglomerular bleeding (10). Glomerular bleeding is indicated by urinary excretion of acanthocytes. Acanthocytes are characteristic ring-formed erythrocytes with vesicle-shaped protrusions (Fig. 1) (11,12) that have been described previously in the peripheral blood of patients suffering from certain hereditary neurological disorders (abetalipoproteinemia, chorea-acanthocytosis, and McLeod syndrome) (13). When urine sediments of patients with biopsy-proven glomerulonephritis and patients with nonglomerular kidney diseases, urolithiasis, cystitis, urethrits, and tumors are compared, acanthocytes are an excellent predictive marker of glomerular bleeding (11).

Acanthocyte formation in glomerulonephritis has been explained by a possible mechanical influence of the impaired glomerular basement membrane on the spectrin backbone of the erythrocyte that passes the glomerular barrier. Compared with glomerulonephritis, diabetic nephropathy is characterized by a different pattern of glomerular lesions, which may result in a different type of hematuria. It has been suggested that hematuria in diabetic nephropathy might result from areas of aneurysmal dilatation in glomerular capillaries with subsequent rupture and with little mechanical damage to erythrocytes.

We studied the prevalence of hematuria and acanthocyturia in patients with clinically diagnosed diabetic nephropathy and in patients with biopsy-proven glomerulonephritis to examine whether acanthocyturia occurs with both glomerular lesions or whether it is specific for glomerulonephritis. In the latter case, the finding of acanthocyturia in a diabetic patient might point to nondiabetic, potentially treatable glomerulopathy, in which renal biopsy may be indicated.
RESEARCH DESIGN AND METHODS — Between April 1999 and August 2001, we examined urine samples of all patients who presented to our Department of Nephrology with the clinical diagnosis of diabetic nephropathy \((n = 68)\) or with biopsy-proven glomerulonephritis \((n = 43)\). Patients with glomerulonephritis were only included if urinalysis was performed before initiation of immunosuppressive treatment of the underlying renal disease. For a control group, urine samples were obtained from 20 volunteers who were matched in age to the patients with the clinical diagnosis of diabetic nephropathy and who had neither symptomatic nephrological or urological diseases nor diabetes.

Diabetic nephropathy was diagnosed in patients treated for type 1 diabetes \((n = 4)\) or type 2 diabetes \((n = 64)\) who were on insulin therapy and who had microalbuminuria \((30–300 \text{ mg albumin/day}; n = 5)\) or overt proteinuria \((>300 \text{ mg protein/day}; n = 63)\). Diabetic nephropathy was not diagnosed in patients in whom case history, renal ultrasonography, urinalysis, or blood chemistry suggested nondiabetic renal disease. No predefined time interval from first diagnosis of diabetes to onset of microalbuminuria was mandatory. The clinical diagnosis of diabetic nephropathy was confirmed by an experienced nephrological consultant.

Patients with diabetic nephropathy and nondiabetic control subjects were older than patients with glomerulonephritis, and renal function was more severely impaired in patients with diabetic nephropathy than in those with glomerulonephritis (Table 1).

Results of renal biopsy in patients with glomerulonephritis included minimal-change disease \((10 \text{ patients})\), IgA nephropathy \((10 \text{ patients})\), mesangial-proliferative glomerulonephritis \((8 \text{ patients})\), membranous glomerulonephritis \((5 \text{ patients})\), focal glomerulosclerosis \((3 \text{ patients})\), crescentic glomerulonephritis \((2 \text{ patients})\), and other forms/mixed forms of glomerulonephritis \((5 \text{ patients})\).

**Urinalysis**

Urinalysis within 4 hours after voiding was performed by an observer who was blinded for the clinical diagnosis and who was experienced in urinanalysis (performing >100 urinary examinations per month). Whenever possible, three urine samples from three separate days were analyzed; this procedure is known to increase sensitivity to detect glomerular bleeding (11).

The urine samples were analyzed by phase-contrast microscopy. Cell numbers of urinary erythrocytes and leukocytes were counted in a Fuchs-Rosenthal counting chamber without prior centrifugation (unspun urine).

For patients with erythrocyte counts \(\geq 8 \text{ red cells/\mu l} \text{ urine (defined as hematuria)}\), red cell morphology was assessed in a spun urine specimen. A total of 10 ml of urine was centrifuged at 2,000 rpm for 5 min. The sediment was resuspended with 0.5 ml of urine. Aliquots of 20 \(\mu l\) of the suspension were analyzed by phase-contrast microscopy to examine at least 100 erythrocytes.

Acanthocytes were defined as a ring-form of erythrocytes with vesicle-shaped protrusions (Fig. 1) (11). Acanthocyturia of \(\geq 5\%\) \((5 \text{ acanthocytes among 100 excreted red cells})\) was classified as glomerular hematuria; acanthocyturia of 2–4% was experienced in urinalysis (performing an observer who was blinded for the clinical diagnosis and who was experienced in urinanalysis).

**Table 1—Biometric data and serum creatinine levels of patients with diabetic nephropathy and patients with glomerulonephritis; biometric data of control subjects**

<table>
<thead>
<tr>
<th></th>
<th>Sex (male/female)</th>
<th>Age (years)</th>
<th>Serum creatinine (\geq 130 \text{ \mu mol/l})</th>
<th>Serum creatinine mean ((\mu \text{mol/l}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic nephropathy ((n = 68))</td>
<td>35/33</td>
<td>64.6 ± 11.7</td>
<td>84% ((57/68))</td>
<td>329.9 ± 213.9</td>
</tr>
<tr>
<td>Glomerulonephritis ((n = 43))</td>
<td>27/16</td>
<td>49.2 ± 16.7</td>
<td>47% ((20/43))</td>
<td>165.1 ± 130.4</td>
</tr>
<tr>
<td>Control subjects ((n = 20))</td>
<td>7/13</td>
<td>56.8 ± 23.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Diabetic nephropathy vs. glomerulonephritis</td>
<td>NS</td>
<td>—</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>Diabetic nephropathy vs. control subjects</td>
<td>NS</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Glomerulonephritis vs. control subjects</td>
<td>NS</td>
<td>—</td>
<td>(P &lt; 0.01)</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are means ± SD, unless otherwise indicated.

Figure 1—Glomerular hematuria characterized by the presence of \(\geq 5\%\) acanthocytes (ring-formed erythrocytes with vesicle-shaped protrusions) among all erythrocytes excreted (phase-contrast microscopy).
Acanthocyturia in diabetic nephropathy

Table 2—Incidence of hematuria and acanthocyturia in patients with diabetic nephropathy, patients with glomerulonephritis, and control subjects upon examination of a single urine sample

<table>
<thead>
<tr>
<th></th>
<th>Hematuria</th>
<th>Acanthocyturia</th>
<th>Hematuria with ≥5% acanthocytes (glomerular hematuria)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(≥8 red cells/μl urine)</td>
<td>Hematuria with ≥2% acanthocytes</td>
<td></td>
</tr>
<tr>
<td>Diabetic nephropathy (n = 68)</td>
<td>62% (42/68)</td>
<td>9% (6/68)</td>
<td>4% (3/68)</td>
</tr>
<tr>
<td>Glomerulonephritis (n = 43)</td>
<td>84% (36/43)</td>
<td>44% (19/43)</td>
<td>40% (17/43)</td>
</tr>
<tr>
<td>Control subjects (n = 20)</td>
<td>20% (4/20)</td>
<td>10% (2/20)</td>
<td>0% (0/20)</td>
</tr>
<tr>
<td>Diabetic nephropathy vs. glomerulonephritis</td>
<td>P = 0.018</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Diabetic nephropathy vs. control subjects</td>
<td>P = 0.002</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Glomerulonephritis vs. control subjects</td>
<td>P &lt; 0.001</td>
<td>P = 0.001</td>
<td>P &lt; 0.001</td>
</tr>
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Data are % (n), unless otherwise indicated.

was considered suspected glomerular hematuria (11). In addition, the urine sample was screened for casts and leukocytes.

Proteinuria was determined by 24-h urine collection in 57 of 68 patients with diabetic nephropathy (84%) and in 32 of 43 patients with glomerulonephritis (74%). In patients who were not compliant with 24-h urine collection, 24-h protein excretion was calculated by means of the total protein-to-creatinine ratio of a single urine specimen, which correlates closely with daily protein excretion measured by 24-h collection (24-h protein excretion [g/day] = urinary protein concentration [mg/l]/[urinary creatinine concentration [mg/dl] · 10]) (14). None of the control subjects showed proteinuria on semiquantitative dipstick examination. Subsequently, 24-h urine collection was not performed in the control subjects.

Statistics

The statistical software Prism 3.00 (GraphPad, San Diego, CA) was used for data management. Frequency counts were compared using Fisher’s exact test. Continuous data are reported as mean ± SD and compared using either the Kruskal-Wallis test followed by Dunn’s post test, or the Mann-Whitney U test (for parameters that were not recorded among control subjects, i.e., serum creatinine level and proteinuria).

RESULTS — Hematuria of ≥8 erythrocytes/μl urine was a frequent finding in patients with glomerulonephritis (84% of all patients upon examination of a single urine sample). Surprisingly, hematuria was also very common in patients with diabetic nephropathy (62% of all patients; P = 0.018 compared with glomerulonephritis) (Table 2). When analyzing three consecutive urine samples, the incidence of hematuria increased to 95% in patients with glomerulonephritis and to 82% in patients with diabetic nephropathy (Table 2). Upon analysis of three urine samples, the prevalence of acanthocyturia increased when examining three urine samples: to 80% (acanthocyturia ≥2%) and 75% (acanthocyturia ≥5%) of all patients with glomerulonephritis (Table 3).

In contrast, acanthocyturia was a rare finding in diabetic nephropathy. In a single sample, acanthocyturia ≥2% was detected in 9% of patients (P < 0.001 compared with patients with glomerulonephritis), and acanthocyturia ≥5% was present in 4% of patients (P < 0.001 compared with patients with glomerulonephritis) (Table 2). Upon analysis of three urine samples, the prevalence of acanthocyturia remained much lower in patients with diabetic nephropathy than in those with glomerulonephritis (P < 0.001) (Table 3).

Among healthy control subjects, acanthocyturia ≥2% was found in 10% (single urinalysis) to 20% (three urine samples). No control subjects had acanthocyturia ≥5%.

There was no significant difference in the prevalence of casts between patients with diabetic nephropathy and patients

Table 3—Incidence of hematuria and acanthocyturia in patients with diabetic nephropathy, patients with glomerulonephritis, and control subjects upon examination of three urine samples

<table>
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<tr>
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<th>Hematuria</th>
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<th>Hematuria with ≥5% acanthocytes (glomerular hematuria)</th>
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<tbody>
<tr>
<td></td>
<td>(≥8 red cells/μl urine)</td>
<td>Hematuria with ≥2% acanthocytes</td>
<td></td>
</tr>
<tr>
<td>Diabetic nephropathy (n = 28)</td>
<td>82% (23/28)</td>
<td>21% (6/28)</td>
<td>11% (3/28)</td>
</tr>
<tr>
<td>Glomerulonephritis (n = 20)</td>
<td>95% (19/20)</td>
<td>80% (16/20)</td>
<td>75% (15/20)</td>
</tr>
<tr>
<td>Control subjects (n = 20)</td>
<td>30% (6/20)</td>
<td>10% (2/20)</td>
<td>0% (0/20)</td>
</tr>
<tr>
<td>Diabetic nephropathy vs. glomerulonephritis</td>
<td>NS</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Diabetic nephropathy vs. control subjects</td>
<td>P &lt; 0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Glomerulonephritis vs. control subjects</td>
<td>P &lt; 0.001</td>
<td>P = 0.002</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Data are % (n), unless otherwise indicated.
with glomerulonephritis (data not shown). Upon a single urinary examination, leukocyturia (>8 leukocytes/µL urine) was found in 42 of 68 patients with diabetic nephropathy and in 30 of 43 patients with glomerulonephritis (NS). Mean proteinuria was 3.8 ± 5.4 g/day in patients with diabetic nephropathy and 4.7 ± 4.4 g/day in patients with glomerulonephritis (NS).

When comparing diabetic patients with acanthocyturia and those without acanthocyturia, there was no significant difference in mean age, type of diabetes, renal function, and prevalence of leukocyturia or casts between both groups. Proteinuria was more pronounced in patients with diabetic nephropathy presenting with acanthocyturia than in those without acanthocyturia (5.3 ± 3.1 g/day vs. 3.6 ± 5.5, respectively; *P* = 0.04). However, the total number of patients with diabetic nephropathy and acanthocyturia was small (*n* = 6).

**CONCLUSIONS** — In reports based on cross-sectional, consecutive series of renal biopsies in unselected diabetic patients with proteinuria, between 23% (8) and 32–33% (7,15) of patients are found to have nondiabetic glomerular disease (in most cases, glomerulonephritis). Because there is general consensus not to perform renal biopsy in all diabetic patients with proteinuria, it is important to define clinical and laboratory features that may indicate the presence of nondiabetic glomerulopathies in diabetic patients and to selectively perform renal biopsy in these patients. The histologic diagnosis of a nondiabetic nephropathy may allow a specific therapeutic approach, such as the initiation of immunosuppressive medication in selected cases.

Among various clinical and laboratory features that have been proposed to indicate nondiabetic nephropathy in diabetic patients, a sudden increase in proteinuria, rapidly progressive renal failure (16), and renal failure in patients without macroalbuminuria, as well as the absence of retinopathy in macroalbuminuric patients (17).

In addition, it has been claimed (1) that hematuria suggests nondiabetic glomerulopathy in diabetic patients. However, hematuria is a rather frequent finding in diabetic patients with proteinuria (1–3,7,8,18,19). More important, in renal biopsy studies in patients with type 2 diabetes and proteinuria, hematuria had either a low specificity for the presence of a nondiabetic nephropathy (7) or its prevalence was not at all increased in nondiabetic nephropathy when compared with isolated diabetic nephropathy (8,9). Therefore, the presence of hematuria does not generally indicate nondiabetic nephropathy, and patterns of hematuria in diabetic patients must be studied in more detail before urinalysis findings may help detect nondiabetic nephropathy.

To our best knowledge, this study presents the first data on urinary erythrocyte morphology in patients with clinically diagnosed diabetic nephropathy. Although hematuria was not discriminative between glomerulonephritis and diabetic nephropathy in our study, we found that the urinary excretion of acanthocytes is a rare finding in the latter. Glomerular hematuria (hematuria comprising ≥5% acanthocytes of all red cells excreted) was seen in only 4% (one urine sample) to 11% (three urine samples) of all patients with diabetic nephropathy.

Contrarily, among all patients with glomerulonephritis, glomerular hematuria was found in 40% (one urine sample) and 75% (three urine samples), which is quite in agreement with frequencies of 52% (one urine sample) and 84% (three urine samples) reported in an earlier study (11).

Our study is limited by the fact that diabetic nephropathy was diagnosed clinically, not histologically, which is in accordance with previous studies on the prevalence of hematuria in patients with diabetic nephropathy (1,4,18). Therefore, in some diabetic patients with albuminuria, glomerulonephritis (either superimposed on diabetic nephropathy or without diabetic nephropathy) may have been overlooked and the underlying renal disease subsequently falsely been classified as (isolated) diabetic nephropathy. However, by neglecting nondiabetic renal diseases in some diabetic patients, we would have overestimated the prevalence of acanthocyturia in diabetic nephropathy. Such misclassification and the prevalence of glomerulonephritis in the general population of ~3% may account for the 4–11% prevalence of glomerular hematuria among patients with the clinical diagnosis of diabetic nephropathy.

Finally, we report a fairly high prevalence of hematuria among our patients with diabetic nephropathy, 62% of whom had hematuria upon a single urinalysis. A similar prevalence of hematuria was found in several cross-sectional studies among patients with overt proteinuria and type 2 diabetes (2,19) or both types of diabetes (3), of whom 21–58% (19), 67–72% (2), and 73% (3) had hematuria. Lower rates of hematuria were reported in patients with type 1 diabetes and the clinical diagnosis of diabetic nephropathy (1,4,18), of whom 12.5% (1) to 35% (18) had hematuria, and in patients with type 2 diabetes and biopsy-proven diabetic nephropathy without superimposed glomerulonephritis, of whom 15% (8) to 35% (9) had hematuria.

First, neither the technique to detect hematuria nor the definition of hematuria is standardized (20). Most studies on hematuria in diabetic nephropathy measured urinary erythrocytes semiquantitatively by direct examination of the centrifuged urinary sediment (sediment count) (1,8,18) and defined hematuria as the excretion of two to three erythrocytes per high-power field (1,8) or even more (4,18). However, current guidelines acknowledge that the determination of the number of erythrocytes per microliter of urine without prior centrifugation (hematuria count) has a greater precision and sensitivity than the sediment count (21). In addition, the same guidelines state that there is no safe lower limit for urinary erythrocytes excretion and that the common definition of hematuria as the excretion of two or three erythrocytes per high-power field may have a limited sensitivity for detection of significant diseases (21). Therefore, we measured the degree of hematuria using the chamber count as the gold standard method, and we defined hematuria as an erythrocyte count of ≥8 red cells/µL, corresponding to about one erythrocyte per high-power field in a centrifuged urine sample. Thus, we chose a very sensitive cutoff value, which allowed us to not miss the presence of significant abnormalities at the price of a lower specificity.

Second, the discrepancy in the prevalence of hematuria partly results from differences in the populations of diabetic patients examined. Studies reporting a prevalence of hematuria <40% in patients with type 1 diabetes and the clinical diagnosis of diabetic nephropathy (1,4,18) or in patients with type 2 diabetes and biopsy-proven diabetic nephropathy
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without superimposed glomerulonephritis (7–9) included patients with a mean age between 17.5 (18) and 57 years (7) and a mean serum creatinine between 88 (18) and 168 μmol/l (4). Additionally, some of these studies included only patients with type 1 diabetes (1,4,18), and some studies included only diabetic patients visiting outpatient clinics (1). In contrast, we present data of patients who were older (mean age 64.6 ± 11.7 years) and had more severely impaired renal function (mean creatinine 329.9 ± 213.9 μmol/l) than in previous studies. The ratio of type 1 to type 2 diabetes was similar to the ratio found in general practice (about 1:10), and we included patients who were admitted to the hospital for complications of diabetes (46% of all patients studied) as well as patients visiting outpatient clinics (54% of all patients).

To our best knowledge, no epidemiologic study has addressed the sources of asymptomatic nonglomerular hematuria in diabetic patients. In addition, there are currently no studies comparing the performance of various diagnostic modalities in the evaluation of nonglomerular hematuria in diabetic patients, and no guidelines have been issued. Because diabetes increases the risk of urinary tract malignancies, diabetic patients with asymptomatic nonglomerular hematuria should undergo urological evaluation of the urinary tract, which is in accordance with recent recommendations (20) and guidelines (21) for the general population.

In summary, hematuria is a frequent finding in patients with diabetic nephropathy, whereas acanthocyturia is rare in this condition, pointing to a nondiabetic renal disease. Therefore, microscopy of the urine sediment should be part of the noninvasive diagnostic workup of diabetic patients with proteinuria to identify diabetic patients with hematuria who are likely to have a nondiabetic glomerulonephritis. We suggest that renal biopsy should be considered when a diabetic patient with proteinuria shows acanthocyturia ≥5% in at least one of three urine samples taken on three different days.

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References