Urinary Protein and Essential Hypertension in Black and in White People

Rajani Chelliah, Giuseppe A. Sagnella, Nirmala D. Markandu, Graham A. MacGregor

Abstract—The objectives of this work were to examine the association between urinary protein and blood pressure and to compare the pattern of urinary protein excretion with essential hypertension in people of European origin (whites) and in people of African or African-Caribbean origin (blacks) living in southwest London, United Kingdom. In the groups as a whole, there were no significant differences in total urinary protein excretion between blacks and whites (geometric means [95% CI]: 94.0 [85.9 to 102.9] mg/24h for the blacks [n=151] and 102.1 [96.1 to 108.4] mg/24h for the whites [n= 219]). There were also no significant differences between blacks and whites in urinary albumin (6.5 [4.9 to 8.5] mg/24h for the blacks [n=97] and 7.1 [5.6 to 9.0] mg/24h for the whites [n=123]). In both groups, those with essential hypertension displayed a significantly raised urinary protein excretion (1.21-fold higher for the blacks and 1.19-fold higher for the whites) and albumin excretion (1.69-fold higher for the blacks and 2.40-fold higher for the whites). Urinary transferrin excretion measured in a subgroup of 67 subjects was also raised in those with essential hypertension (3.22-fold higher in the blacks and 2.76-fold higher in the whites). Examination of urinary proteins by SDS-PAGE did not identify any pattern consistent with a reduction in renal tubular protein reabsorption in those with essential hypertension. These results suggest that the increase in protein excretion in essential hypertension could be due, at least in part, to an increase in glomerular protein ultrafiltration. (Hypertension. 2002;39:1064-1070.)

Key Words: ethnicity ■ proteinuria ■ albuminuria ■ renal disease ■ cardiovascular diseases ■ hypertension, essential

Microalbuminuria (MA), defined as urinary albumin excretion of 30 to 300 mg/24h, is a well-recognized major risk factor for the development of overt proteinuria, nephropathy, and vascular disease in people with diabetes mellitus.1,2 However, it is now apparent that an increase in urinary albumin excretion is also associated with an increased risk of cardiovascular disease independent of the presence of diabetes.1–3

MA is also associated with essential hypertension. However, widely different prevalence rates, ranging from 5% to 37%, have been reported, and not every study has demonstrated a direct association with blood pressure levels.1–6 Nevertheless, the presence of MA and proteinuria in people with essential hypertension is an independent risk factor for end-organ damage and renal failure.3–7 The development of renal failure is of particular relevance in people of African or Afro-Caribbean origin (blacks),8,9 and some studies have suggested that MA in blacks may reflect a greater susceptibility to renal damage from relatively smaller increases in blood pressure.10,11

Although in normal people albumin is the major urinary protein excreted,12,13 it is not clear whether increased albumin excretion in people with essential hypertension is also associated with raised excretion of other proteins. This is important because the pattern of protein excretion may provide further insight into the renal defects leading to increased protein excretion.7,14 Because of this, the objectives of this work were to examine the association between urinary protein and blood pressure and to compare the pattern of urinary protein excretion in relation to essential hypertension in black people with that in white people.

Methods

Subjects and Blood Pressure Measurements

This study examined 370 subjects, of whom 151 were of African or Afro-Caribbean origin (blacks) and 219 of European origin (whites). There were 121 normotensives (46 blacks: 19 female, 27 male; 75 whites: 31 female, 44 male) and 249 subjects with essential hypertension (105 blacks: 52 female, 53 male; 144 whites: 49 female, 95 male). Normotensives had blood pressures less than 140 mm Hg for systolic and 90 mm Hg for diastolic. Those with essential hypertension had blood pressures greater than 140 mm Hg for systolic and 90 mm Hg for diastolic. Individuals with clinical or biochemical evidence of secondary hypertension, diabetes, renal failure, or overt proteinuria (urinary protein >1 g/24h) were excluded. All patients had either never received treatment for hypertension or had withdrawn from treatment 3 weeks earlier. The study was approved by the local Ethical Committee. All were studied in the morning, and all were on their normal sodium intake.

Each subject’s weight and height was recorded and, after a 5-minute rest, blood pressure (mean of 3 readings) was determined using an OMRON HEM-705CP (Omron Corporation) in the supine or sitting position. Blood was taken for routine biochemical mea-
urements and 24-hour urinary collections were made for electrolytes, creatinine, and urinary protein excretion. All samples for urinary protein measurements were immediately frozen and stored at –20°C.

**Measurement of Total Urinary Protein**

Total urinary protein was measured using a dye-binding method (pyrogallol red-molybdate complex) as described by Watanabe et al with minor modifications. The coefficients of variation were 3.9% (intra-assay, n=15) and 4.7% (inter-assay, n=15).

**Gel Electrophoresis of Urinary Protein**

SDS-PAGE electrophoresis of urinary protein was carried out on urinary protein extracts precipitated with pyrogallol red-molybdate reagent before electrophoresis essentially as described by Marshall et al. Individual urine samples (500 μL) were mixed with 1 mL of pyrogallol red-molybdate reagent and centrifuged for 5 to 10 minutes to collect the precipitated protein. With this method the recovery of total protein from the urine was greater than 80%.

Individual pellets from the precipitates were resublimed in 100 μL of Laemmlı denaturing, reducing buffer (NOVEX), heated at 85°C (2 minutes), centrifuged (2 minutes), and subjected to electrophoresis on commercial 4% to 20% Tris-glycine SDS-PAGE gradient gels (8cm phoresis on commercial 4% to 20% Tris-glycine SDS-PAGE gradient gels (8cm phoresis on commercial 4% to 20% Tris-glycine SDS-PAGE gradient gels) in a NOVEX XCell II Mini-Cell electrophoresis tank at room temperature according to the manufacturer’s instructions (NOVEX Electrophoresis GmbH). After completion of the run, the gel was removed carefully from the cassette and stained with 50% v/v methanol, 10% v/v acetic acid containing 0.05% wt/vol Coomassie Brilliant Blue R-250, and destained in aqueous 5% v/v methanol, 7% acetic acid. The gels were dried using the Dry-Ease kit (NOVEX Electrophoresis GmbH).

**Measurement of Urinary Transferrin**

The urinary concentration of transferrin was assayed by a double-antibody sandwich ELISA using commercially available reagents based on a modification of the method described by Bang et al. Rabbit anti-human transferrin (Cat No A0061), used as the capture antibody; horseradish peroxidase (HRP), conjugated to rabbit anti-human transferrin (Cat No PE563) as the detection antibody; and OPD tablets (1,2-phenylenediamine dichloride; Cat No S2045) were obtained from DAKO Ltd. The standard transferrin (multiprotein lytes, creatinine, and urinary protein excretion. All samples for urinary protein measurements were immediately frozen and stored at –20°C.

**Results**

Demographic characteristics, blood pressures, and laboratory measurements for the normotensive and hypertensive groups are summarized in Table 1. Average (geometric means; 95% confidence intervals) urinary protein excretion in the white group was 102.1 (96.1–108.4) mg/24h and 94.0 (85.9–102.9) mg/24h in the black group (difference not significant). In both blacks and whites, urinary protein excretion was significantly higher in the hypertensive groups (Table 1). Urinary protein was still significantly higher in the hypertensive groups even after adjustment for age, gender, body mass index (BMI), and urinary creatinine excretion or creatinine clearance.

In either the normotensive or in the hypertensive group of white people analyzed separately, there were no significant associations between the level of systolic or diastolic blood pressure and urinary protein excretion. However, there was a significant association in the combined group of whites between urinary protein and systolic blood pressure (r=0.15; P=0.02) but not with diastolic blood pressure (r=0.07). Similar results were obtained for the blacks (combined group: r=0.27 for systolic [P=0.001] and r=0.18 for diastolic blood pressure [P=0.02]). However, given the considerable scatter, it is also apparent that these associations are relatively weak (Figure 1).

The results from the logistic analyses confirmed statistically significant associations between hypertension and urinary protein in the white people. This association remained significant even after adjusting for age, gender, BMI, and urinary creatinine excretion (β coefficient [SE]=1.04±0.38; odds ratio [OR]=2.82; P=0.01; n=201). There was also a positive association in the blacks, but in this case the regression coefficient did not reach statistical significance after adjustment for age, gender, BMI, and urinary creatinine excretion (β coefficient [SE]=0.54±0.42; OR=1.71; P=0.21; n=130).

The results for urinary albumin were in line with those found for total urinary protein in that there were no significant differences in urinary albumin excretion between blacks (6.5; 4.9–8.5 mg/24h; n=97) and whites (7.1; 5.6–9.0 mg/24h; n=123). Urinary albumin was significantly raised in the whites with essential hypertension (3.8; 2.6–5.4 mg/24h; n=35, for the normotensive versus 9.1; 6.9–12.1 mg/24h; n=88, for the hypertensive group). Average urinary albumin was also higher in the group of black hypertensives (4.5; 2.6–7.8 mg/24h; n=29, for the normotensive versus 7.6; 5.6–10.3 mg/24h; n=68, for the hypertensive group). The correlation coefficients between urinary albumin and systolic or diastolic blood pressure were of similar magnitude to those found for total urinary protein (r=0.18 to 0.32).

**Pattern of Urinary Protein Excretion**

In view of the considerable individual variability in the proportion of albumin relative to total protein excretion (Figure 2) further investigations were carried out to examine...
the pattern of urinary protein excretion and in particular to assess whether there were any differences between normotensives and hypertensives. This was carried out by high resolution protein separation on SDS-PAGE on a total of 90 individual samples (30 blacks and 60 whites). Samples from normotensives and hypertensives were matched for total urinary protein concentration to minimize any confounding effects caused by major differences in basal urinary protein excretion.

Typical patterns of protein separation are illustrated in Figure 3. When the patterns of protein bands from normotensives (n=25) were compared (by visual examination) with those from hypertensives (n=65), it became apparent that a higher proportion of the hypertensives (52% versus 83%; \( P<0.002 \)) displayed a strong band in the region corresponding to a molecular weight of approximately 77 kDa (Figure 3). Further work on the identification of the protein within this band suggested identity with urinary transferrin.
Measurement of Urinary Transferrin

Urinary transferrin was measured by a specific ELISA in a subgroup of 67 individuals. Demographic characteristics, blood pressures, and other laboratory measurements for this subgroup were comparable to those of the main groups (Table 2). There were no significant differences between blacks (220.9; 136.4–357.9 μg/24h; n = 28) and whites (257.6; 172.7–384.2 μg/24h; n = 39). However, urinary levels were significantly higher in the hypertensive groups in both blacks and whites (Table 2, Figure 4). These differences were maintained even after adjustment for age, gender, BMI, and urinary creatinine (P = 0.02). In the combined group of normotensives and hypertensives, urinary transferrin was significantly associated with blood pressure in the whites (r = 0.51, P = 0.001 for systolic and r = 0.36, P = 0.025 for diastolic blood pressure) and in the blacks (r = 0.38; P = 0.048 and r = 0.49, P = 0.008 for systolic and diastolic blood pressures, respectively).

**Table 2. Demographic Characteristics, Blood Pressures, Biochemical Measurements, and Urinary Transferrin Excretion in a Subgroup of 67 Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Whites Normotensives</th>
<th>Essential Hypertensives</th>
<th>Blacks Normotensives</th>
<th>Essential Hypertensives</th>
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<tbody>
<tr>
<td><strong>Demographics and blood pressures</strong></td>
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<tr>
<td>Age, y</td>
<td>50.2 ± 12.1 (12)</td>
<td>48.6 ± 15.2 (27)</td>
<td>43.4 ± 12.3 (11)</td>
<td>48.1 ± 9.9 (17)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>73.2 ± 10.7 (11)</td>
<td>82.3 ± 17.6 (27)</td>
<td>76.9 ± 12.6 (11)</td>
<td>77.7 ± 9.7 (17)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.9 ± 2.6 (11)</td>
<td>27.9 ± 4.9 (26)</td>
<td>26.2 ± 3.5 (11)</td>
<td>27.3 ± 2.9 (16)</td>
</tr>
<tr>
<td>Systolic, mm Hg</td>
<td>124.1 ± 12.9 (12)</td>
<td>161.3 ± 19.1 (27)</td>
<td>126.4 ± 10.5 (11)</td>
<td>160.5 ± 14.5 (17)</td>
</tr>
<tr>
<td>Diastolic, mm Hg</td>
<td>78.7 ± 7.1 (12)</td>
<td>99.1 ± 11.8 (27)</td>
<td>79.1 ± 6.0 (11)</td>
<td>98.4 ± 9.9 (17)</td>
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<tr>
<td><strong>Serum biochemistry</strong></td>
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<tr>
<td>Sodium, mmol/L</td>
<td>138.7 ± 2.6 (12)</td>
<td>139.5 ± 3.1 (27)</td>
<td>139.0 ± 1.6 (11)</td>
<td>139.8 ± 2.3 (17)</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>4.1 ± 0.17 (11)</td>
<td>4.2 ± 0.28 (27)</td>
<td>4.2 ± 0.34 (10)</td>
<td>4.1 ± 0.26 (16)</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>85.0 ± 15.3 (11)</td>
<td>86.9 ± 12.3 (27)</td>
<td>91.5 ± 19.4 (11)</td>
<td>86.5 ± 17.3 (17)</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.1 ± 0.94 (11)</td>
<td>5.1 ± 0.59 (26)</td>
<td>4.8 ± 0.55 (11)</td>
<td>5.4 ± 0.96 (15)</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>43.5 ± 2.5 (10)</td>
<td>46.2 ± 4.1 (25)</td>
<td>44.8 ± 2.9 (9)</td>
<td>45.5 ± 3.1 (13)</td>
</tr>
<tr>
<td>Total protein, g/L</td>
<td>71.4 ± 5.6 (10)</td>
<td>73.4 ± 4.2 (23)</td>
<td>77.4 ± 5.3 (10)</td>
<td>76.8 ± 2.8 (14)</td>
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<tr>
<td><strong>Urinary measurements</strong></td>
<td></td>
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<tr>
<td>Volume, mL/24 h</td>
<td>2255 ± 1173 (12)</td>
<td>2025 ± 678 (27)</td>
<td>1397 ± 562 (11)</td>
<td>1705 ± 618 (17)</td>
</tr>
<tr>
<td>Sodium, mmol/24 h</td>
<td>152.3 ± 55.5 (12)</td>
<td>140.9 ± 68.8 (26)</td>
<td>134.5 ± 84.8 (10)</td>
<td>147.0 ± 33.6 (16)</td>
</tr>
<tr>
<td>Potassium, mmol/24 h</td>
<td>87.5 ± 35.8 (12)</td>
<td>81.3 ± 28.8 (26)</td>
<td>69.0 ± 26.9 (10)</td>
<td>78.7 ± 37.6 (16)</td>
</tr>
<tr>
<td>Creatinine, mmol/24 h</td>
<td>12.5 ± 2.7 (12)</td>
<td>13.5 ± 3.4 (27)</td>
<td>16.7 ± 5.9 (10)</td>
<td>14.2 ± 3.51 (16)</td>
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<tr>
<td>Creatinine clearance, mL/min</td>
<td>101.7 ± 22.4 (11)</td>
<td>107.9 ± 22.3 (27)</td>
<td>124.4 ± 35.8 (10)</td>
<td>116.6 ± 35.3 (16)</td>
</tr>
<tr>
<td>Transferrin, μg/24 h</td>
<td>127.4 (73.2–221.6) (12)</td>
<td>352.3 (217.9–569.4) (27)</td>
<td>108.6 (67.6–174.3) (11)</td>
<td>349.8 (182.7–669.7) (17)</td>
</tr>
</tbody>
</table>

Group values are mean ± SD (n); urinary transferrin given as geometric mean (95% confidence interval).

*P = 0.01–0.05 (normotensives vs hypertensives).
In this study we have compared urinary protein excretion in blacks and in whites and examined the pattern of urinary protein excretion in relation to hypertension in people with apparently normal renal function without overt proteinuria. Although MA and proteinuria have been found repeatedly in blacks with overt diabetes and nephropathy,18–22 few studies have directly compared urinary protein excretion between blacks and whites with normal renal function.

In agreement with previous studies of white people, we have found that urinary protein excretion was significantly raised in those with essential hypertension. Urinary protein was also raised in the blacks with essential hypertension, but there was no significant difference in urinary protein excretion between blacks and whites. This lack of difference between blacks and whites was observed in both the normotensive groups and in the hypertensive groups and could not be attributed to any differences in age or gender, nor to any differences in urinary creatinine or creatinine clearance. There were no significant differences in blood pressure between the blacks and the whites, and moreover, examination of the association between blood pressure and urinary protein excretion also did not provide any evidence suggesting a greater sensitivity of urinary protein excretion to increasing blood pressure in the blacks. Similarly, there were also no significant differences in urinary albumin excretion between blacks and whites. By contrast, other studies have reported a higher urinary albumin in blacks compared with whites. In a population-based study of young adults, Jiang et al10 found higher urinary albumin in young African-American blacks compared with whites and a significant (though relatively weak) association with blood pressure in the African-Americans, but not in American whites. These observations, therefore, may not apply to older people with hypertension, but in a separate investigation, Summerson et al11 compared adult African-American blacks and whites with essential hypertension and found that urinary albumin in the blacks was nearly twice as high as that in the whites. The lack of significant differences in urinary protein (or albumin) excretion between blacks and whites in our study is not due to sample size limitations because the power of our study to detect differences of even 50% in total protein or albumin was >80%. However, it is important to note that there are differences in study design (eg, overnight urinary collections versus 24-hour urinary collections for albumin measurement, severity of hypertension, etc). Clearly, other factors and, in particular, the presence and duration of diabetes and impaired glucose metabolism may also be important. A much higher prevalence of proteinuria has been reported in blacks with long-standing noninsulin-dependent diabetes mellitus (NIDDM) and hypertension.18–22 By contrast, a lower prevalence has been observed in blacks with newly developed NIDDM.23 Our results, in people with apparently normal renal function and without overt diabetes, therefore, do not necessarily refute the possibility of an increasingly greater susceptibility to renal damage in black people with increasing blood pressure and with the development of diabetes.

Although proteinuria has generally been regarded as a marker of the severity of underlying disease, more recent evidence now suggests that proteins filtered through the glomerular capillaries can have an intrinsic renal toxicity that might well contribute to the progression of renal damage and eventual renal failure.7,14

One way to gain some insight into tubular mechanisms for protein reabsorption is to examine the pattern of the different proteins excreted.24,25 In the present study, this was done using SDS-PAGE, a method which allows the separation of proteins according to their molecular weights. As expected, a large number of proteins with a wide range of molecular sizes were visualized (Figure 3), but we were not able to identify any major pattern consistent with a reduction in tubular protein reabsorption in those with hypertension compared with normotensive controls. However, inspection of the pattern of proteins on an individual basis demonstrated that samples from people with essential hypertension displayed greater frequency of a band corresponding to urinary transferrin, suggesting a higher urinary excretion of this protein. That this was the case was confirmed by direct measurement of urinary transferrin because significantly raised levels were found in the groups with essential hypertension for both ethnic groups (Figure 4).

Although urinary transferrin is only slightly larger than albumin (≈77 versus 65 kDa), it is less anionic than albumin (isoelectric point 5.7 versus 4.9), and work in diabetes has suggested that a raised urinary transferrin precedes abnormally raised urinary albumin.26–28 Few studies have been carried out on the measurement of urinary transferrin in people with hypertension. One study in diabetic patients demonstrated that, although diabetics were 3 times as likely as controls to have elevated urinary transferrin excretion, this was not associated with coexisting hypertension.29 However, Cheung et al30 did report a significant association between urinary transferrin and blood pressure in diabetics. Moreover, and consistent with our observation, Bang et al17 also reported significantly raised urinary excretion of transferrin in white people with essential hypertension. Interestingly, the present study also suggests a stronger association between blood pressure and urinary transferrin in both blacks and whites compared with total protein or albumin. However, given that measurements of urinary transferrin were done on a smaller subset, these observations need to be confirmed on a larger sample. Moreover, whether urinary transferrin could be used as an index of subsequent, more severe renal impairment in people with essential hypertension cannot be answered because this would require long-term prospective investigations.

The higher albumin and transferrin excretion and a lack of evident tubular proteinuria clearly suggest that the high protein excretion in people with essential hypertension is more than likely secondary to an increase in intraglomerular pressure and/or impairment of the glomerular basement membrane.

The impact of systemic blood pressure on intraglomerular hemodynamics depends not only on the systemic pressure but also on changes in the tone of the afferent and efferent arterioles, which, in turn, are important determinants of filtration force. A stronger association with systolic blood pressure might be expected because this dictates the pressure...
load on the glomerulus unless counteracted by preglomerular vasoconstriction. However, despite the significantly higher urinary protein in the hypertensive group, the association between blood pressure levels and urinary protein or albumin excretion in both blacks and whites was relatively weak (r<0.32) and similar in magnitude for both systolic and diastolic pressures. Although other investigations suggest stronger associations between albumin excretion and ambulatory blood pressure, potentially a better index of pressure load, other factors (eg, impairment in intraglomerular hemodynamics and/or changes in glomerular membrane permeability), to some extent, may also be important determinants for the higher protein excretion.

**Perspectives**

Since the original report of a higher rate of transcapillary albumin exchange in subjects with hypertension nearly 25 years ago, there has been considerable interest in the significance of urinary protein excretion in people with essential hypertension. However, it is now apparent that an increased urinary albumin excretion is also associated with cardiovascular disease. This has led to the hypothesis that MA may reflect systemic transvascular protein leakiness, possibly mediated by endothelial dysfunction. The association between raised urinary protein excretion and a generalized endothelial dysfunction is especially important because other studies have demonstrated a positive association between direct measurement of fractional disappearance rates of albumin from the plasma compartment and urinary albumin excretion in clinically healthy people. Moreover, also in clinically healthy people, a slightly elevated urinary albumin excretion was associated with impaired arterial dilatory capacity. These results, in conjunction with the recent observation that the risk of cardiovascular disease associated with raised urinary albumin appears to be continuous with increasing albumin excretion with no evidence of a threshold level, point to urinary protein excretion as an important marker of cardiovascular risk.

**Acknowledgments**

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**References**

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