except for serum urea, and low serum urea persisted after discharge.

When we compared the reagents among the three urea measurement methods, the low urea value was not generated at color development but at the urease reaction step. Possible enzyme reaction inhibitors include (*a*) substrates, products, and compounds structurally similar to substances or products; (*b*) inhibitors of metalloenzymes; (*c*) reagents acting on the sulfhydryl group; (*d*) heavy metals; and (*e*) other drugs and antibodies against enzymes *(5)*. An inhibitor of urease of any origin, hydroxamic acid, and a specific inhibitor, acyl phosphate, have been described *(1)*. After IgG in the patient's serum was adsorbed by protein A *(6)*, a urea value similar to that detected by the jack bean method was obtained by the bacterial method, suggesting that IgG was bound to the bacterial urease and inhibited enzyme activity. Because the labeled antibody used in the ELISA method was polyclonal and protein A can adsorb multiple types of IgG, with the exception of IgG3, whether the bound IgG was monoclonal or polyclonal is unknown. Although details of the mechanism are unclear, one possibility is that the patient previously was exposed to bacterial urease and developed IgG that formed antigen-antibody complex with the urease in the reagent.

Various bacterial enzymes are used in many test reagents, including a *Proteus* species-derived urease in an NADPH-dependent urea reagent. We conclude that it is necessary to consider enzyme inhibition by immunoglobulin in test reagents containing bacterial enzymes.

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Urinary Glycosaminoglycan Excretion in Newly Diagnosed Essential Hypertensive Patients, *Dilek Yavuz,[1]* Ahmet Toprak,[2] Yasemin Budak,[3] H. Önder Ersöz,[1] Oğuzhan Deyneli,[1] Hakan Tezcan,[2] and Sema Akalin[1]* (Marmara University School of Medicine, [1] Section of Endocrinology and Metabolism and [2] Department of Cardiology, Sakizgulu sok. No. 1-3, D:15, Kadikoy Istanbul, Turkey 81030; [3] Hipokrat Research Laboratories, Istanbul, Turkey; * author for correspondence: fax 90-216-428-0013, e-mail dyavuz@turk.net)

Glycosaminoglycans (GAGs) are major components of the basement membranes and play a key role in their molecular organization and function *(1–3)*. Some authors have proposed that increased loss of proteoglycan from glomerular basement membrane (GBM) alters glomerular charge selectivity, which contributes to urinary loss of albumin *(4–8)*.

Several clinical studies have shown that the GAG content of human GBM is significantly decreased and that urinary loss of GAG is markedly increased in diabetic patients *(7–9)*. Among the possible mechanisms of diabetic microalbuminuria are decreased synthesis *(10)* and/or increased loss of GAGs *(9, 11)* from the GBM.

Although the importance of microalbuminuria is unclear in hypertensive patients, it may be an early marker of glomerular functional and structural changes *(12, 13)*. Because hypertension is considered a systemic disease, basement membrane changes should be widespread. Whether basement membranes from other tissues are also affected and whether these changes are the result of hypertension remains to be determined. Therefore, in our study, we examined the effects of hypertension on basement membrane anionic charges in newly diagnosed, untreated essential hypertensive patients.

Twelve nonsmoking, essential hypertensive patients, stage I or II according to the sixth report of Joint National Committee *(14)*, and 12 age- and sex-matched healthy normotensive controls were included in the study. All patients gave informed consent, and the institutional local ethics committee approved the study.

Blood pressure was measured after a 30-min rest on three different occasions at the sitting position, and Korotkov sounds phase V were estimated as diastolic blood pressure. None of the patients had ever received any antihypertensive medication before participation in the study. The duration of the hypertension was 6 ± 4 months according to the medical records. Secondary hypertension was excluded. Diabetes and impaired glucose tolerance were excluded with an oral glucose tolerance test.

A 24-h urine sample was collected, and creatinine, microalbumin, total GAG, and GAG subfractions were measured. Blood samples were also collected to measure red blood cell anionic charge (RBCCh) and creatinine concentrations.

RBCCh was evaluated with a cationic dye, Alcian Blue 8Gx (cat. no. A5268; Sigma) according to a previously described method *(15, 16)*, with minor modifications. Platelets and leukocytes were removed by the method of Beutler et al. *(17)*. The intra- and interassay CVs for Alcian Blue binding were 5.8% and 7.6%, respectively.

The urine total GAG concentration was measured in 24-h urine samples with a colorimetric method described by Jong et al. *(18)*, using 1,9-dimethylene blue (Aldrich) and bovine kidney heparan sulfate as the calibrator (cat. no. H 7640; Sigma) with a Shimadzu 2000 UV spectrophotometer at a wavelength of 520 nm. The intra- and interassay CVs were 2.4% and 15%, respectively.

Urinary GAG subfractions were separated with a method described by Heickendorff et al. *(19)* with minor modifications. In untreated urine samples, GAGs were

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precipitated with cetylpyridinium chloride after an overnight incubation at 4 °C and then centrifuged. GAG complexes were then dissolved in *n*-propanolol. Ethanol containing 20 g/L potassium acetate was used for further precipitation; distilled water was added to dissolve the complexes. Isolated GAGs were separated by electrophoresis on cellulose acetate (Titan III; Helena Laboratory). The samples were analyzed in duplicate using 0.1 mol/L barium acetate (pH 5.0) buffer and 0.3 mol/L cadmium acetate (pH 4.1) buffer systems. The distribution of GAGs was quantified by densitometry at a wavelength of 610 nm after staining with 1 g/L Alcian Blue. The following were used as calibrators: heparan sulfate from bovine kidney (cat. no. H 7640; Sigma), chondroitin sulfate A from bovine trachea (cat. no. C 8529; Sigma), and dermatan sulfate from shark cartilage (cat. no. C 4384; Sigma). The distribution of GAG types was expressed as fraction of the total GAG content.

Urinary albumin was measured by the nephelometric method, using a kit from Behring Diagnostics. The inter- and intraassay CVs were 4.4% and 4.3%, respectively. The urine serum and creatinine concentrations were measured by the automated Jaffé method with a Boehringer kit.

Statistical analysis was performed with an IBM-compatible PC using the Instat II program. Kruskal-Wallis ANOVA, Mann–Whitney U-tests, and Student *t*-tests were used as appropriate for comparisons, and the Spearman rank test was used for correlation analysis. The results were expressed as mean ± SE.

The clinical and biochemical findings in both groups are shown in Table 1. The urinary total GAG excretion was significantly higher in the hypertensive patients ($P < 0.05$). Although the heparan sulfate subfraction was higher in hypertensive patients compared with the normotensive group, the dermatan sulfate subfraction of GAG was higher in the normotensives. Urinary chondroitin sulfate subfractions were similar in both groups. However, when absolute urinary excretion rates were considered, the excretion rates for heparan, chondroitin, and dermatan sulfate in urine were 3.68, 1.22, and 4.58 mg/day for the hypertensive group, and 0.35, 2.06, and 2.8 mg/day for the control group, respectively.

The binding of Alcian Blue to RBCs was 448 ± 6.3 ng Alcian Blue/10^6 RBC in hypertensive patients and 468 ± 1.5 ng Alcian Blue/10^6 RBC in the normotensive group ($P < 0.001$). Although the urinary albumin excretion rate was negatively correlated with RBCCh ($r = -0.35$; $P < 0.05$), it did not reach statistical significance with urinary total GAG excretion. RBCCh was slightly correlated with urinary total GAG excretion ($r = -0.43$; $P < 0.05$). Diastolic blood pressure was positively correlated with albuminuria ($r = 0.59$; $P < 0.005$) and urinary total GAG excretion ($r = 0.55$; $P < 0.01$).

Similar increases of 24-h urinary total GAG excretion have been reported in diabetic patients. Previous studies have found that an increased urinary heparan sulfate excretion rate is associated with the loss of basement membrane anionic charge in diabetic nephropathy *(7–10)*. If hypertension is considered a systemic disease that exhibits vascular dysfunctional changes and end-organ complications similar to those seen in diabetes *(12, 13)*, increased urinary GAG excretion could be attributed to the renal effects of hypertension.

Because RBCCh, which is a crude reflection of GBM anionic charge *(15, 20)*, was correlated with urinary GAG excretion in our study, we propose an association between increased urinary GAG excretion and the loss of GBM anionic content in essential hypertensive patients. Heintz et al. *(21)* documented a significantly decreased urinary small heparan sulfate excretion in hypertensive patients that is clearly distinct from basement membrane-associated large heparan sulfate proteoglycan. Taken together, these findings suggest a complex rearrangement of GAG metabolism in hypertension, with both decreased synthesis and/or increased urinary loss of local heparan sulfate-containing molecules that may contribute to the former.

Our study showed that all GAG components other than heparan sulfate were affected in the early stages of essential hypertension.

The correlation between diastolic blood pressure and albuminuria has been shown previously *(22, 23)*. In our study, diastolic blood pressure values were positively correlated with 24-h urinary albumin and GAG excretion, suggesting a direct effect of increased arterial pressure on GBM. We conclude that hypertension alters urinary GAG

Table 1. Clinical characteristics and biochemical findings in hypertensive patients and healthy controls.

Characteristic	Hypertensive group (n = 12)	Control group (n = 12)	*P*
Age, year	50.6 ± 9.8	46 ± 10.3	NS[a]
Sex ratio, women/men	10/2	9/3	
Body mass index, kg/m^2	24.5 ± 0.8	25.9 ± 0.6	NS
Systolic blood pressure, mmHg	168.0 ± 14.9	122.7 ± 12.9	<0.05
Diastolic blood pressure, mmHg	97.5 ± 2.6	76.0 ± 1.8	<0.0001
Serum creatinine, mg/L	8.3 ± 0.5	8.9 ± 0.5	NS
Creatinine clearance, mL/min	103.6 ± 11.1	97.1 ± 8.0	NS
Microalbuminuria, mg/day	51.2 ± 20.1	14.0 ± 3.1	<0.05
Urinary 24-h total GAG excretion, mg/day	9.49 ± 1.54	5.31 ± 0.88	<0.05
Urinary heparan sulfate subfraction, % of total GAGs	38.8 ± 4.3	6.6 ± 1.0	<0.0001
Urinary dermatan sulfate subfraction, % of total GAGs	12.9 ± 3.7	39.1 ± 2.6	<0.0001
Urinary chondroitin sulfate subfraction, % of total GAGs	48.3 ± 2.5	54.4 ± 2.3	NS
RBCCh, ng Alcian Blue/ 10^6 RBC	448.1 ± 6.3	468.5 ± 1.5	<0.001

[a] NS, not significant.

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excretion and that loss of glomerular anionic content may be associated with increased urinary GAG excretion.

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Prenatal Diagnosis of Myotonic Dystrophy Using Fetal DNA Obtained from Maternal Plasma, *Paola Amicucci,[1,2] Massimo Gennarelli,[3] Giuseppe Novelli,[1,2*] and Bruno Dallapiccola[1,2]* ([1] Department of Biopathology and Diagnostic Imaging, Tor Vergata University of Rome, Via Di Tor Vergata 135, 00133 Rome, Italy; [2] CSS-Mendel, Piazza Galeno 3, 00161 Rome, Italy; [3] Istituto di Ricovero e Cura a Carattere Scientifico, Fatebenefratelli, Via Pilastroni 4, 25125 Brescia, Italy; * author for correspondence: fax 39-06-20427313, e-mail novelli@med.uniroma2.it)

Myotonic dystrophy (DM; MIM 160900) is an autosomal dominant disorder associated with expansion of an unstable CTG trinucleotide repeat in the 3′ untranslated region of the DM kinase gene (*DMPK*) on chromosome 19q13 *(1)*. Patients are heterozygous for expanded alleles in the range of 50–4000 repeats *(1)*. The molecular diagnosis of DM routinely is performed by analyzing the CTG number on genomic DNA extracted from various biological sources, including trophoblast cells sampled at 10–11 weeks of amenorrhea during the first trimester of pregnancy *(2, 3)*. We evaluated the possibility of using maternal plasma for prenatal diagnosis of DM, by monitoring the pregnancy of an unaffected woman whose husband was affected by DM (70 CTG repeats).

All participants gave oral and written informed consent.

A blood sample (~10 mL) was collected at 10 weeks of gestation before chorionic villus sampling (CVS) and was centrifuged at 3000*g* for 10 min. Plasma was carefully removed from EDTA-containing tube and centrifuged again at 3000*g* for 10 min. DNA was then extracted from 2 mL of the centrifuged plasma with a QIAamp Blood Kit (Qiagen). The elution volume of the final step was 300 μL. Genomic DNA was also extracted from chorionic villi and peripheral blood lymphocytes of both parents.

To check for the presence of fetal DNA in maternal plasma, we performed microsatellite DNA analysis (CSF1PO) and Y-specific PCR (amelogenin) amplification after having ascertained that the fetus was a male (Fig. 1, A and B). *DMPK* CTG repeat amplification was carried out as reported previously *(2)* with a slight modification. A first round of PCR consisting of 15 cycles (30 s at 94 °C, 1 min at 62 °C, 5 min at 68 °C, and a final elongation of 5 min at 68 °C), was performed in 30 μL of reaction mixture, using 25 pmol each of forward and reverse primers DMK9003 (5′-CACAGGCTGAAGTGGCAGTTCCA-3′) and DMK11111 (5′-TGTCGGGGTCTCAGTGCATCCA-3′) *(2)*, and 5–10 μL of the extracted DNA. We reamplified 1 μL of this first-round reaction, using 25 pmol each of forward and reverse primers MDY-1D (5′-GCTCGAAGGGTCCTTGTAGCCG-3′) and MDY-Z2A (5′-TTCCCGAGTAAGCAGGCAGA-3′) *(3)* for 40 additional cycles, using the same cycling and reaction conditions. Amplicons were separated by 1% agarose gel electrophoresis and blotted onto a nylon membrane. Filters were hybridized with $(CTG)_5$ ^{32}P-labeled oligonucleotide as described *(3)*. The same protocol was used for genomic

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