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Role of Transforming Growth Factor-β_2 in, and a Possible *Transforming Growth Factor-β_2* Gene Polymorphism as a Marker of, Renal Dysfunction in Essential Hypertension: A Study in Turkish Patients

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ABSTRACT

Background: Many studies have shown that transforming growth factor (TGF)-β has a major role in renal scarring in many renal diseases and hypertension.

Objectives: The primary aim of this study was to investigate both the relationship between hypertension and serum and urinary levels of TGF-β_2 (a more sensitive isoform for glomeruli than TGF-β_1), and the effects of combination therapy with perindopril + indapamide on microalbuminuria, which becomes an early indicator of hypertensive benign nephropathy, and serum and urinary TGF-β_2 levels in patients with mild to moderate essential hypertension. In addition, we examined the possible relationship between *TGF-β_2* gene polymorphism and essential hypertension.

Methods: This study was conducted at the Department of Nephrology, Medical Faculty, Gazi University, Ankara, Turkey. Patients aged ≥18 years with newly diagnosed mild to moderate essential hypertension (systolic/diastolic blood pressure [SBP/DBP] >120/>80 mm Hg) who had not previously received antihypertensive treatment were included in the study. Patients with stage I hypertension received perindopril 2 mg + indapamide 0.625 mg (tablet), and patients with stage II hypertension received perindopril 4 mg + indapamide 1.125 mg (tablet). All study drugs were given OD (morning) PO with food for 6 months. Serum and urinary TGF-β_2 and creatinine levels and serum and urinary albumin levels were measured before and after perindopril + indapamide administration. Amplified DNA fragments of the TGF-β_2 primer region were screened using amplification refractory mutation system polymerase chain reaction analysis, and the number of ACA repeats was confirmed by DNA sequencing. Genetic studies were performed using a commercial TGF-β_2 kit.

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Results: Forty patients were enrolled in the study, and 38 patients (27 women, 11 men; mean [SD] age, 46.3 [6.5] years) completed it. SBP and DBP were significantly decreased from baseline with perindopril/indapamide (both, $P < 0.001$). Microalbuminuria and urinary TGF-β_2 levels also decreased significantly from baseline ($P = 0.04$ and $P < 0.001$, respectively), whereas the serum TGF-β_2 level did not change significantly. Three patients, all of whom were found to have *TGF-β_2* gene mutations, had increased urinary TGF-β_2 levels despite good blood pressure control.

Conclusions: The results of this study in patients with mild to moderate hypertension suggest that, despite good clinical control of blood pressure, the persistence of microalbuminuria and high urinary TGF-β_2 levels might predict renal impairment. When treating these patients, genetic tendencies and possible polymorphisms on the *TGF-β_2* locus should be kept in mind. (*Curr Ther Res Clin Exp.* 2005;66:266–278)

Key words: hypertension, perindopril, indapamide, microalbuminuria, TGF-β_2, gene polymorphism.

INTRODUCTION

Rapid deterioration of renal function and other renal abnormalities (eg, fibrinoid and necrotizing changes in parenchymal and vascular structures) are seen in some patients with uncontrolled hypertension.[1] Renal lesions associated with benign hypertension have been termed *benign nephrosclerosis*.[2]

Microalbuminuria becomes an important clinical indicator of renal damage in patients with hypertension.[3] However, it is a weak predictor of renal disease progression in nondiabetic patients with hypertension. In fact, aggressive control of hypertension is required in the presence of albuminuria.[4,5] Even though microalbuminuria can be helpful in predicting renal disease, more evidence is needed for the assessment of hypertensive benign nephrosclerosis in predicting renal disease.

Some growth factors might play a role in the progression of renal scarring in hypertensive patients. Two studies[6,7] have suggested that transforming growth factor (TGF)-β has a significant role in renal scarring in different renal diseases and hypertension. Thus, either serum or urinary TGF-β levels might reflect the progression of this scarring. Although both the TGF-β_1 and TGF-β_2 isoforms are expressed throughout the kidney, TGF-β_2 and its mRNA are found mainly in the glomeruli.[8] A study of antibodies against 3 isoforms[9] showed that TGF-β_1 and TGF-β_3 were present in the tubule cells, whereas staining for TGF-β_2 was intense in the glomeruli and faint in the tubules. Several previous studies[10–12] have reported a strong correlation between *TGF-β_1* DNA polymorphisms, hypertension, and hypertension-related end-organ damage.

Because of generally inadequate compliance with dietary sodium restriction in the Turkish population, physicians tend to begin antihypertensive treatment with a low-dose diuretic/antihypertensive combination.[13,14]

The primary aim of this study was to investigate the relationship between hypertension and serum and urinary levels of TGF-β_2 (a more sensitive isoform for glomeruli compared with TGF-β_1), and the effects of combination therapy with the angiotensin-converting enzyme inhibitor perindopril plus the diuretic indapamide on microalbuminuria and serum and urinary TGF-β_2 levels in patients with mild to moderate essential hypertension. In addition, we examined the possible relationship between *TGF-β_2* gene polymorphism and essential hypertension.

PATIENTS AND METHODS

This study was conducted at the Department of Nephrology, Medical Faculty, Gazi University, Ankara, Turkey. The study protocol was approved by the ethics committee at the university (Gazi University Medical Faculty) retrospectively, because that committee was not formed until after the study was completed.

Inclusion and Exclusion Criteria

Turkish patients aged ≥18 years with newly diagnosed mild to moderate essential hypertension (systolic/diastolic blood pressure [SBP/DBP] >120/>80 mm Hg) who were seen in an outpatient clinic were recruited for the study. The authors believe these patients were representative of the general Turkish population in terms of socioeconomic and cultural status.

Exclusion criteria included the presence of secondary hypertension (renal failure, rheumatoid diseases, pregnancy, endocrine causes) or diabetes mellitus or a history of the use of any antihypertensive drug or any other drug that might affect renal function.

All patients were informed of the study design and provided written informed consent to participate.

Measurements

To rule out secondary hypertension at screening, routine blood biochemistry tests (including fasting plasma glucose [FPG] level, blood urea nitrogen [BUN] level, and serum concentrations of creatinine [SCC], total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], very-low-density lipoprotein cholesterol, triglycerides [TG], total protein, albumin, uric acid, sodium, potassium, calcium, and phosphorus), urinalysis, thyroid function tests, creatinine clearance (CrCl), chest radiography, electrocardiography, and abdominal ultrasonography were performed. Serum albumin and serum and urinary TGF-β_2 levels were measured. To measure TGF-β_2 levels, pretreatment 24-hour urine and blood samples were collected and stored at −70°C, according to kit recommendations (Human TGF-beta 2 DuoSet Economy Pack, 45 Plate, Quantikine, R&D Systems, Inc., Minneapolis, Minnesota). Possible correlations between microalbuminuria and basic study parameters (SBP/DBP and BUN, SCC, and serum and urinary TGF-β_2) were inves-

tigated. The presence of microalbuminuria was assessed using nephelometry (Turbox Immunochemistry System, Orion Diagnostica, Espoo, Finland). Values >16.6 mg/L were considered as microalbuminuria.

For all patients, blood pressure was measured in both arms in the sitting and supine positions using a standard hand-grip sphygmomanometer after at least 15 minutes of rest. The mean of 3 measurements was used.

Staging of hypertension was determined based on the recommendations in the Sixth Report of the Joint National Committee.[15] Patients were instructed to return to the outpatient clinic for monthly follow-up for 6 months. Follow-up consisted of a history of patient observations (eg, efficacy, adverse effects [AEs]); physical examination, including blood pressure measurements; and blood biochemistry. Patients with high serum lipid levels (TC >200 mg/dL or TG >200 mg/dL) were initially treated with atorvastatin 10 mg PO QD (evening).

Patients with stage I hypertension received perindopril 2 mg + indapamide 0.625 mg (tablet), and patients with stage II hypertension received perindopril 4 mg + indapamide 1.125 mg (tablet). All study drugs were given QD (morning) PO with food for 6 months. Patients with stage I hypertension whose blood pressure had not normalized were switched to the higher dose of perindopril + indapamide.

At the end of the 6-month treatment period, all of the assessments were repeated and 24-hour urine and blood samples were again collected to measure TGF-β_2 levels. Samples were thawed at room temperature and assessed using an enzyme-linked immunosorbent assay kit (Quantikine Human TGF-β_2 Immunoassay, R&D Systems, Inc.).

Genetic studies were performed using a commercial TGF-β_2 kit (R&D Systems, Inc.) human polymerase chain reaction (PCR) TGF-β_2 primer pair (catalog number RDP-27). First, DNA was extracted from peripheral blood (5 mL collected into EDTA) by the standard method, with proteinase K digestion followed by phenol/chloroform extraction. The TGF-β_2 primer region was as follows: 5′-CGA CGA CAA CGA TGA TGC TT-3′ and 5′-TAC GTA CAG CAA CAA CTC CAC TT-3′. Amplified DNA fragments of the TGF-β_2 primer region were screened by amplification refractory mutation system–PCR analysis, and the number of ACA repeats was confirmed using DNA sequencing. Alleles *1, 2,* and *3* of the gene contained 7, 8, and 9 ACA repeats, respectively. The *TGF-β_2* gene had an ACA repeat sequence in its 3′-noncoding region, and polymorphisms were dependent on differences in ACA repeats.[16] PCR amplification was carried out in 50-µL volumes of reaction mixtures containing 75 mM tris-hydrochloride (pH 8.8), 200 mM $(NH_4)\ 2SO_4$, 0.1% Tween-20, 2.0 mM $MgCl_2$, 50 mM of each deoxyribonucleotide triphosphate, 50 mM of previously reported[16] specific primers, 1 U Taq DNA polymerase (Promega Corporation, Madison, Wisconsin), and a 0.2- to 0.5-mg DNA sample. All PCR reactions were processed at 94°C for 4 minutes, followed by 30 cycles at 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 45 seconds; the final extension at 72°C for 10 minutes was performed in an automated thermal cycler (Perkin Elmer Cetus 9600, Applied Biosystems, Foster City,

California). The amplified products were analyzed using electrophoresis on 1.5% agarose gel.

Tolerability was assessed using patient interview.

Statistical Analysis

Descriptive statistics are expressed as mean (SD) for parametric data or median (range) for nonparametric data. The Student *t* test and Wilcoxon signed rank test were used to compare means of groups. Correlations were evaluated using the Pearson and Spearman bivariate correlation tests. The Kruskal-Wallis test was used to analyze between-group differences. $P < 0.05$ was considered statistically significant.

RESULTS

Forty patients entered the study, and 2 of them dropped out (1 moved to another city and 1 was lost to follow-up). Thirty-eight patients (27 women, 11 men; mean [SD] age, 46.3 [6.5] years) completed the study (**Table I**). At baseline, mean (SD) SBP/DBP was 165.1 (8.1)/103.4 (6.6) mm Hg. Eight of 38 patients had stage I hypertension and were treated with perindopril 2 mg + indapamide 0.625 mg/d, and 30 had stage II hypertension and were treated with perindopril 4 mg + indapamide 1.25 mg/d. By the end of the first month of treatment, 2 patients with stage I hypertension were switched to the higher dose of perindopril + indapamide because their blood pressure had not normalized.

Table I. Baseline demographic and clinical characteristics of the study group (N = 38).

Characteristic	Value
Demographic	
Age, mean (SD), y	46.3 (6.5)
Sex, no.	
Female	27
Male	11
Clinical	
Body mass index, mean, kg/m^2	27.1
Concomitant medications, no.	
Cholesterol lowering	14
Hypertension stage, no.	
I*	8
II†	30

*These patients were assigned to receive perindopril 2 mg + indapamide 0.625 mg.

†These patients were assigned to receive perindopril 4 mg + indapamide 1.25 mg.

Fourteen patients received atorvastatin during the study for the treatment of hyperlipidemia.

Clinical findings and biochemical parameters at baseline and after 6 months of treatment are shown in **Table II**. After 6 months of antihypertensive therapy, mean SBP/DBP was significantly reduced from baseline (change, 42.4/19.8 mm Hg, respectively; both, $P < 0.001$). Significant decreases from baseline were seen in microalbuminuria (median [range], 6 [1–150] vs 5 [1–42] mg/L; $P = 0.04$) and urinary TGF-β_2 level (median [range], 16.4 [0.87–204.5] vs 6.3 [0.87–21.9] pg/mL; $P < 0.001$), whereas the change in serum TGF-β_2 level was not significant (median [range], 32.8 [1.9–294.6] vs 1.9 [1.9–255.0] pg/mL).

When the data for each patient were examined individually, we found that urinary TGF-β_2 levels were decreased in 31 patients, increased in 3 patients, and did not change significantly in 4 patients, despite good blood pressure control.

At baseline and after 6 months of treatment, SBP/DBP and serum TGF-β_2, BUN, and SCC did not correlate with the presence of microalbuminuria. However, a

Table II. Comparison of blood pressure and biochemical concentrations before and after 6 months of treatment with perindopril + indapamide for mild to moderate essential hypertension. Values are presented as mean (SD) unless otherwise specified.

Parameter	Before Treatment	After Treatment	P*
SBP, mm Hg	165.1 (8.1)	122.7 (7.0)	<0.001
DBP, mm Hg	103.4 (6.6)	83.6 (3.7)	<0.001
FPG, mg/dL	102.6 (11.7)	99.0 (9.8)	0.03
BUN, mg/dL	14.6 (3.8)	15.6 (3.5)	NS
SCC, mg/dL	0.9 (0.1)	0.9 (0.1)	NS
TC, mg/dL	221.2 (36.6)	205.0 (35.6)	0.002
HDL-C, mg/dL	43.0 (10.9)	48.0 (12.6)	0.003
LDL-C, mg/dL	144.4 (33.6)	132.5 (47.3)	NS
CrCl, mL/min	98.4 (24.1)	102.6 (23.4)	NS
Microalbuminuria, mg/L†	6.0 (1–150)	5.0 (1–42)	0.04
Serum TGF-β_2, pg/mL†	32.8 (1.9–294.6)	1.9 (1.9–255.0)	NS‡
Urinary TGF-β_2, pg/mL†	16.4 (0.87–204.5)	6.3 (0.87–21.9)	<0.001‡

SBP = systolic blood pressure; DBP = diastolic blood pressure; FPG = fasting plasma glucose; BUN = blood urea nitrogen; NS = nonsignificant; SCC = serum creatinine concentration; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; CrCl = creatinine clearance; TGF-β_2 = transforming growth factor-β_2.
*Student *t* test unless otherwise specified.
†Because of nonparametric distribution, values are presented as median (range).
‡Wilcoxon signed rank test.

strong correlation was found between the presence of microalbuminuria and urinary TGF-β_2 level at baseline (Spearman correlation [r_s] = 0.650; P = 0.001) and after 6 months of treatment (r_s = 0.711; P = 0.001) (**Table III**).

When laboratory parameters were analyzed based on the presence of microalbuminuria, no significant between-group differences were found (**Table IV**).

When DNA analysis was performed on individual samples, the samples of 3 patients showed *TGF-β_2* gene polymorphism. These primers amplified between Arg 299 and Arg of the 433 of the unprocessed 442 aa form of the TGF-β_2 protein. The aa encoded by the primers was identical to the sequence of the *TGF-β_2* gene available in the public domain (accession number M19154). Allele *2* of the *TGF-β_2* gene, corresponding to 8 ACA repeats, was common between these 3 patients.

None of the patients complained of treatment-emergent AEs (cough, headache, peripheral vertigo, hyperkalemia, or asthenia) that required changing the treatment protocol. Four (10.5%) patients reported nausea during the first 3 weeks of treatment, but this effect was mild and transient.

DISCUSSION

In many diseases of the kidneys, the progression to end-stage renal disease (ESRD) depends largely on the severity of the damage to both the tubulointerstitial and glomerular compartments. Extensive reduction of renal mass is one of several factors that induce glomerulosclerosis.[17]

Because compliance with a salt-restricted diet is often inadequate in the Turkish population, physicians tend to begin antihypertensive treatment with low-dose combination diuretic therapy.[13,14] We treated hypertension with a combination of perindopril + indapamide because of its well-known effectiveness

Table III. Correlation between microalbuminuria and other parameters before and after 6 months of treatment with perindopril + indapamide for mild to moderate essential hypertension (N = 38).

	Before Treatment		After Treatment	
Parameter	r_s*	P	r_s*	P
SBP	–0.120	NS	0.190	NS
DBP	–0.020	NS	0.010	NS
BUN	–0.088	NS	0.090	NS
SCC	0.005	NS	–0.007	NS
Serum TGF-β_2	0.022	NS	0.247	NS
Urinary TGF-β_2	0.650	0.001	0.711	0.001

r_s = Spearman correlation coefficient; SBP = systolic blood pressure; NS = nonsignificant; DBP = diastolic blood pressure; BUN = blood urea nitrogen; SCC = serum creatinine concentration; TGF-β_2 = transforming growth factor-β_2.
*Because of nonparametric distribution, Spearman correlation was performed.

Table IV. Comparison of parameters based on the presence of microalbuminuria before and after 6 months of treatment with perindopril + indapamide for mild to moderate essential hypertension (N = 38).* Values are presented as mean (SD) unless otherwise specified.

	Microalbuminuria			No Microalbuminuria		
Parameter	Before Treatment	After Treatment	*P*	Before Treatment	After Treatment	*P*
SBP, mm Hg	163.3 (7.5)	126 (7.5)	0.001	165.4 (8.3)	122.0 (6.8)	0.02
DBP, mm Hg	101.6 (9.3)	82.5 (4.1)	0.004	103.7 (6.2)	83.9 (3.7)	0.02
BUN, mg/dL	12.5 (2.7)	15.6 (3.6)	NS	15.0 (3.9)	15.6 (3.8)	NS
SCC, mg/dL	0.9 (0.02)	0.9 (0.01)	NS	0.9 (0.1)	0.9 (0.1)	NS
Serum TGF-β_2†	44.0 (1.9–103.0)	40.3 (1.9–114.5)	NS	27.1 (1.9–294.0)	1.9 (1.9–255.0)	NS
Urinary TGF-β_2†	37.5 (15.5–170.0)	6.4 (1.9–19.2)	0.02	13.2 (0.8–204.5)	6.3 (0.8–21.95)	0.02

SBP = systolic blood pressure; DBP = diastolic blood pressure; BUN = blood urea nitrogen; NS = nonsignificant; SCC = serum creatinine concentration; TGF-β_2 = transforming growth factor-β_2.
*No significant between-group differences were found.
†Evaluated with Kruskal-Wallis test; presented as median (range).

in decreasing intraglomerular pressure.[18] We used indapamide because hydrochlorothiazide promotes growth of vascular smooth muscle.[19]

A significant decrease in blood pressure was achieved by the end of the 6-month treatment period ($P < 0.001$). At the end of the study, FPG level had decreased statistically significantly ($P = 0.03$) (although the decrease was not clinically meaningful), whereas the TC level decreased ($P = 0.002$) and the HDL-C level increased ($P = 0.003$), suggesting that the patients conformed to the recommendations of cholesterol-lowering treatment, diet, and exercise.

Impaired renal function is one of the most important and well-known consequences of uncontrolled hypertension. Angiotensin-converting enzyme inhibition causes vasodilation of efferent arterioles and may cause defects in renal perfusion, particularly in patients with conditions such as congestive heart failure, renal arterial stenosis, and hypovolemia.[20] Although none of the patients in the present study had complicated hypertension, we did not find any decrease in renal function based on SCC or CrCl at baseline or at the end of the study period.

Pathogenetic pathways common to both benign nephrosclerosis and microalbuminuria might suggest that microalbuminuria plays a role in the development of hypertensive benign nephropathy.[5] Although microalbuminuria has been attributed to various mechanisms (ie, functional and/or structural abnormalities of the glomeruli, vessels, and tubuli), the origin of hypertensive microalbuminuria remains obscure.[17] Bigazzi et al[3] and Tsioufis et al[21] showed that microalbuminuria is closely related to impaired arterial elasticity in untreated patients with essential hypertension. The same studies also showed a significant decrease in CrCl, particularly during the first 6-month follow-up in the microalbuminuric group. It could be speculated that controlling microalbuminuria might delay the progression of hypertension-related renal disease for a longer period compared with controlling blood pressure alone.

In the present study, 6 (15.8%) patients had microalbuminuria at baseline; 5 (83.3%) of them had normal albumin levels at the end of the 6-month treatment period. No correlation was found between the presence of microalbuminuria and hypertension staging. This finding suggests that microalbuminuria is not only affected by systemic blood pressure but also by activation of the renin-angiotensin-aldosterone system and increased intraglomerular pressure.[17] We observed a reduction in microalbuminuria as well as good blood pressure control. However, it is uncertain whether this effect would have been achieved with only the combination of perindopril + indapamide or if it was the effect of treatment with an angiotensin-converting enzyme inhibitor. To answer this question, further research of similar design but using other antihypertensive agents should be performed.

Inducing cell growth is only one of the multiple effects that TGF-β exerts on cells. TGF-β has also been shown to mediate mesangial sclerosis after experimental acute glomerulonephritis and will likely be shown to be important in a variety of other renal disorders as well.[9–11]

Five TGF-β isoforms share 64% to 82% homology among their amino acid sequences.[10] TGF-β_1 is widely distributed in renal tissue, with immunohistochemical staining showing the greatest concentration of TGF-β_1 protein in the tubular epithelial cells. With the use of antibodies against each of 3 isoforms, TGF-β_1 and TGF-β_3 have been identified in the tubule cells, whereas TGF-β_2 staining was shown to be most intense in the glomeruli.[10,22,23]

Although the role of TGF-β_1 has been investigated extensively in hypertensive patients[24–26] with ESRD, a literature search (key words: *TGF-β_2*, *renal scarring*, *hypertension*, and *microalbuminuria*; years: 1985–2004) did not identify any studies of TGF-β_2 in these patients. Yu et al[27] assessed the fibrogenic role of TGF-β_2 on renal cells, particularly in glomeruli. They found that each isoform increased matrix protein synthesis and reduced matrix degradation by renal cells similarly, and that TGF-β_2 stimulated TGF-β_1 production.

In our study, serum TGF-β_2 levels varied widely (range, 1.9–294.6 pg/mL) and were not correlated with blood pressure levels. Also, no correlation was found between serum TGF-β_2 levels and microalbuminuria, which becomes an early indicator of hypertensive benign nephrosclerosis. All TGF-β isoforms are widely distributed in tissues other than the kidney, and the serum levels of the isoforms may be affected not only by renal abnormalities but also other pathologic processes, such as atherosclerosis. For this reason, serum levels of TGF-β_2 might not specifically reflect renal damage.

Several trials[6,24,28,29] have shown that urinary cytokine level is a more effective indicator of renal disease progression compared with serum cytokine level. In fact, circulating cytokines might originate outside of the kidneys, and the glomerular filtration rate of these molecules is still unclear. Because cytokines that have a crucial role in the progression of renal fibrosis might be expressed in renal tissue, measuring urinary levels of these molecules might be essential in evaluating the progression of nephrosclerosis.[30,31]

In the patients with microalbuminuria (6/38), the high levels of urinary TGF-β_2 at the beginning of the study (median, 37.5 pg/mL [range, 15.5–170.8 pg/mL]) decreased significantly by the end of the study (median, 6.4 pg/mL [range, 1.9–19.2 pg/mL]; $P = 0.02$), as did SBP/DBP (both, $P < 0.001$). Urinary TGF-β_2 levels were well correlated with microalbuminuria at the beginning and end of the study. These findings were similar to results of previous trials that showed urinary cytokine level to be an early and accurate indicator of renal disease, as was microalbuminuria.[23,29] These data suggest that control of microalbuminuria might delay the progression of hypertension-related renal disease and that urinary TGF-β_2 level and microalbuminuria might be good markers as well. When we analyzed the data according to existing microalbuminuria, results did not show a significant between-group difference. This finding might suggest that urinary TGF-β_2 levels should be examined independent of microalbuminuria in hypertensive patients to detect renal impairment.

Previous studies[16,23] showed multiple polymorphisms for 3 isoforms of the *TGF-β* gene. Freedman et al[32] found that *TGF-β_2* gene polymorphism did not

seem to play a major role in the initiation of renal failure in African Americans. However, Alansari et al[33] defined 2 novel polymorphisms in the *TGF-β_2* gene in 3 white populations in Spain, Turkey, and the United Kingdom that may play a role in diseases associated with the *TGF-β_2* gene. Although we had a small number of patients for a genetic trial, we also tried to determine whether a relationship exists between *TGF-β_2* gene polymorphism and urinary and serum TGF-β_2 levels. Interestingly, 3 patients with increased urinary TGF-β_2 levels despite good blood pressure control and decreased microalbuminuria also had the only 3 *TGF-β_2* gene mutations we found. If the number of patients in the study had been higher, the probability of having samples with mutated genes would have been greater and we could have speculated that these mutations would be predictive of renal disease progression, even in patients with decreased blood pressure and microalbuminuria. Certainly, these 3 samples are not sufficient proof of the genetic tendency of benign nephrosclerosis in our study population. Further research using a large number of patients with hypertensive renal disease is necessary.

This study was planned as a self-controlled trial; the findings would have been more useful if we had also included patients with another condition (eg, diabetes) or a control group for comparison. Another limitation of this study was the necessity of combination treatment. Certainly, we cannot state that the improvement seen in the patients during the study was the result of the treatment modality.

CONCLUSIONS

The results of this study in patients with mild to moderate essential hypertension suggest that, despite good clinical control of blood pressure in these patients, the persistence of microalbuminuria and high urinary TGF-β_2 levels might predict renal impairment. When treating these patients, genetic tendencies and possible polymorphisms on the *TGF-β_2* locus should be kept in mind.

REFERENCES

1. Kaplan NM. *Primary Hypertension: Pathogenesis in Clinical Hypertension.* 7th ed. Baltimore, Md: Lippincott Williams & Wilkins; 1998:41–88.
2. el Nahas AM. Renal scarring: The role of angiotensin II. *Nephrol Dial Transplant.* 1995;10:28–32.
3. Bigazzi R, Bianchi S, Baldari D, Campese VM. Microalbuminuria predicts cardiovascular events and renal insufficiency in patients with essential hypertension. *J Hypertens.* 1998;16:1325–1333.
4. Damsgaard EM, Froland A, Jorgensen OD, Mogensen CE. Microalbuminuria as predictor of increased mortality in elderly people. *BMJ.* 1990;300:297–300.
5. Mikhail N, Fukuda N, Tremblay J, Hamet P. Platelets, growth factors, and vascular smooth-muscle cells in hypertension and diabetes. *J Cardiovasc Pharmacol.* 1993;22 (Suppl 6):S64–S74.

6. Baroni EA, Costa RS, Volpini R, Coimbra TM. Sodium bicarbonate treatment reduces renal injury, renal production of transforming growth factor-beta, and urinary transforming growth factor-beta excretion in rats with doxorubicin-induced nephropathy. *Am J Kidney Dis.* 1999;34:328–337.
7. De Albuquerque DA, Saxena V, Adams DE, et al. An ACE inhibitor reduces Th2 cytokines and TGF–beta1 and TGF–beta2 isoforms in murine lupus nephritis. *Kidney Int.* 2004;66:869. Letter.
8. Daopin S, Piez KA, Ogawa Y, Davies DR. Crystal structure of transforming growth factor-beta 2: An unusual fold for the superfamily. *Science.* 1992;257:369–373.
9. Sharma K, Ziyadeh FN. The transforming growth factor-beta system and the kidney. *Semin Nephrol.* 1993;13:116–128.
10. Border WA, Ruoslahti E. Transforming growth factor-beta in disease: The dark side of tissue repair. *J Clin Invest.* 1992;90:1–7.
11. Sharma K, Ziyadeh FN. The emerging role of transforming growth factor-beta in kidney diseases. *Am J Physiol.* 1994;266:F829–F842.
12. Senior K. The complex puzzle of gene variation and essential hypertension. *Mol Med Today.* 1999;5:506.
13. Ertürk S. Treatment aspect in non-compliant patients, 6. Presented at the National Hypertension & Renal Diseases Congress; June 2–6, 2004; Belek/Antalya, Turkey.
14. Turkish Cardiology Association. *National Guide of Hypertension Treatment and Follow-Up.* Istanbul, Turkey: TCA; 2000.
15. The Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [published correction appears in *Arch Intern Med.* 1998;158:573]. *Arch Intern Med.* 1997;157:2413–2446.
16. Nishimura DY, Purchio AF, Murray JC. Linkage localisation of TGF-β_2 and the human homeobox gene HLX1 to chromosome 1q. *Genomics.* 1993;15:357–364.
17. United Kingdom Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38 [published correction appears in *BMJ.* 1999;318:29]. *BMJ.* 1998;317:703–713.
18. PROGRESS Collaborative Group. Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6,105 individuals with previous stroke or transient ischaemic attack [published corrections appear in *Lancet.* 2001;358:1556 and *Lancet.* 2002;359:2120]. *Lancet.* 2001;358:1033–1041.
19. Hadrava V, Kruppa U, Russo RC, et al. Vascular smooth muscle cell proliferation and its therapeutic modulation in hypertension. *Am Heart J.* 1991;122:1198–1203.
20. Zarif L, Covic A, Iyengar S, et al. Inaccuracy of clinical phenotyping parameters for hypertensive nephrosclerosis. *Nephrol Dial Transplant.* 2000;15:1801–1807.
21. Tsioufis C, Tzioumis C, Marinakis N, et al. Microalbuminuria is closely related to impaired arterial elasticity in untreated patients with essential hypertension. *Nephron Clin Pract.* 2003;93:c106–c111.
22. Ray PE, McCune B, Gomez RA, et al. Induction of transforming growth factor-beta 2-3 in the juxtaglomerular apparatus and renal vascular smooth muscle cells of young rats and infants. *Exp Nephrol.* 1994;2:129. Letter.
23. Burrow CR. Regulatory molecules in kidney development. *Pediatr Nephrol.* 2000;14: 240–253.
24. Cambien F, Ricard S, Troesch A, et al, for the Etude Cas-Temoin de l'Infarctus du Myocarde (ECTIM) Study. Polymorphisms of the transforming growth factor-beta 1 gene in relation to myocardial infarction and blood pressure. *Hypertension.* 1996;28:881–887.

25. Li B, Khanna A, Sharma V, et al. TGF-beta1 DNA polymorphisms, protein levels, and blood pressure. *Hypertension.* 1999;33:271–275.
26. August P, Suthanthiran M. Transforming growth factor beta and progression of renal disease. *Kidney Int Suppl.* 2003;87:S99–S104.
27. Yu L, Border WA, Huang Y, Noble NA. TGF-beta isoforms in renal fibrogenesis. *Kidney Int.* 2003;64:844–856.
28. Pfeilschifter J, Pignat W, Leighton J, et al. Transforming growth factor beta 2 differentially modulates interleukin-1 beta- and tumour-necrosis-factor-alpha-stimulated phospholipase A2 and prostaglandin E2 synthesis in rat renal mesangial cells. *Biochem J.* 1990;270:269–271.
29. Pfeilschifter J, Vosbeck K. Transforming growth factor beta 2 inhibits interleukin 1 beta- and tumor necrosis factor alpha-induction of nitric oxide synthase in rat renal mesangial cells. *Biochem Biophys Res Commun.* 1991;175:372–379.
30. London GM, Asmar RG, O'Rourke ME, Safar ME. Improvement in large artery mechanical properties with the very low dose perindopril/indapamide combination—The Reason Project. In: Program and abstracts of the 11th European Meeting on Hypertension; June 15–19, 2001; Milan, Italy. Abstract PS 20/193.
31. Wong W, Singh AK. Urinary cytokines: Clinically useful markers of chronic renal disease progression? *Curr Opin Nephrol Hypertens.* 2001;10:807–811.
32. Freedman BI, Yu H, Spray BJ, et al. Genetic linkage analysis of growth factor loci and end-stage renal disease in African Americans. *Kidney Int.* 1997;51:819–825.
33. Alansari A, Hajeer AH, Bayat A, et al. Two novel polymorphisms in the human transforming growth factor beta 2 gene. *Genes Immun.* 2001;2:295–296.

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