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ORIGINAL ARTICLE

Impact of cytokine gene polymorphism on cardiovascular risk in renal transplant recipients

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Summary

Cardiovascular events are the leading causes of morbidity and mortality in renal transplant recipients (RTR). Given the role of inflammation in atherosclerosis, the contribution of functional polymorphisms of cytokines to cardiovascular diseases (CVD) was assessed in RTR in this study. Polymorphisms of tumour necrosis factor alpha (TNF-α) gene [−308 (G → A), −238 (G → A)], interleukin-10 (IL-10) gene [−1082(A → G), −819 (T → C), −592 (A → C)], transforming growth factor beta 1 (TGF-β1) gene [codon 10 (T → C), codon 25 (G → C)], carotis intima media thickness (CIMT), left ventricular mass index (LVMI), 24-h ambulatory blood pressure and serum lipoprotein homocysteine level, erythrocyte sedimentation rate, serum C-reactive protein (CRP) and serum fibrinogen level of RTR were determined. Seventy-two RTR (26 cadaveric allograft, 46 living-related allograft, 43 male, 29 female) were included in this study. LVMI were similar in TNF-α, IL-10 and TGF-β1 genotypes. Right and left CIMT were higher in TT genotype ($n = 16$) than CT ($n = 46$) and CC ($n = 10$) genotypes of TGF-β1 codon 10 (T → C) gene polymorphism (RCIMT, 7.7 ± 2.2 mm vs. 7.0 ± 1.4 mm vs. 5.9 ± 1.4 mm, $P = 0.025$; LCIMT, 8.5 ± 2.5 mm vs. 7.0 ± 1.3 mm vs. 6.1 ± 1.2 mm, $P = 0.002$). Lipoprotein (a) level of TT genotype (35.5 ± 22.5 mg/dl) was higher than CC (4.1 ± 2.8 mg/dl) and CT (20.4 ± 11.2 mg/dl) genotypes of TGF-β1 codon 10 (T → C) gene polymorphism ($P = 0.037$). High producers of cytokine IL-10 −1082 [GG ($n = 22$) vs. AA + AG ($n = 50$)] and low producers of TGF-β codon 25 [GC + CC ($n = 17$) vs. GG ($n = 55$)] had lower IMT of carotid artery but the difference did not reach statistical significance ($P > 0.05$). The CIMT of renal transplant patients was similar in IL-10 (−819, −592) and TNF-α (−308, −238) genotypes. No difference was observed in 24-h ambulatory blood pressure levels, serum lipoproteins, plasma homocysteine level, erythrocyte sedimentation rate, serum CRP, serum fibrinogen level in IL-10, TNF-α and TGF-β1 genotypes. Besides the well-known factors, TGF-β1 gene polymorphisms might play a role in CVD in RTR even at early stages of asymptomatic atherosclerosis.

Introduction

Cardiovascular diseases (CVD) are the major causes of morbidity and mortality in renal transplant recipients (RTR) [1]. Given the fatal cardiovascular events in recipients with functioning grafts, CVDs have also become an important cause of graft loss. The high incidence of CVD in RTR mainly relates to atherosclerosis and left ventricular hypertrophy (LVH). High-resolution B-mode ultrasonography is a noninvasive technique for assessing the asymptomatic carotid atherosclerosis and has proved to be a useful predictive marker for the identification of subjects at high risk for cardiovascular events [2]. An increase in the prevalence of carotid lesions and close association of the asymptomatic carotid atherosclerosis with cardiovascular events has been reported in renal transplant patients [3]. On the contrary, echocardiographically provided LVH proved to be an useful prognostic marker with respect to subsequent cardiac death [4].

Experimental and clinical studies based on markers of inflammation in plasma, as well as in atherosclerotic tissue samples, have provided evidence for the presence of ongoing inflammation in atherosclerosis [5]. Proinflammatory cytokines such as tumour necrosis factor alpha (TNF-α), transforming growth factor beta 1 (TGF-β1), interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-10 (IL-10) contribute to the activation of endothelial cells and the modulation of macrophage and vascular smooth muscle cell function. Similar to atherosclerosis, activation of cytokines such as IL-6, IL-1, TGF-β and TNF-α by haemodynamic overload has been also implicated in the pathogenesis of LVH. These cytokines exacerbate haemodynamic imbalances, act as negative inotropes, stimulate LVH and promote the production of more cytokines [6].

The proinflammatory cytokine TNF-α contributes to atherosclerosis by activating growth factors, cytokines and adhesion molecules. The presence of TNF-α in the majority of atherosclerotic lesions suggests its involvement in the pathogenesis of atherogenesis [5]. Polymorphisms within TNF-α gene locus at positions −308 (G → A) and −238 (G → A) in the promoter region have higher rate of transcription of cytokines [7]. TNF-α gene polymorphism is associated with metabolic syndrome, myocardial infarction and calcified coronary lesions [8,9].

Interleukin-10 is a well-known anti-inflammatory cytokine, produced by Th2 lymphocytes, B cells and monocytes [10]. It inhibits macrophage-dependent cytokine synthesis by Th1 cells and regulates the balance between cell- and humoral-mediated immune responses. IL-10 may arrest and even reverse the chronic inflammatory response in established atherosclerosis both *in vivo* and *in vitro* [11]. The best documented IL-10 gene promoter polymorphisms with higher transcriptional level of cytokine are −1082 (A → G), −819 (T → C) and −592 (A → C) [12]. IL-10 gene polymorphisms have a predictive role in short- and long-term outcomes of renal transplant patients [13].

Transforming growth factor beta is a multifunctional key regulatory cytokine in atherosclerosis. TGF-β stimulates extracellular matrix production such as collagen and fibronectin and inhibits the proliferation and migration of vascular smooth muscle in culture media [5]. T to C transition at nucleotide 29 and G to C transition at nucleotide 74 in the regions encoding the signal sequence, which results in substitutions of Leu to Pro at codon 10 and Arg to Pro at 25, respectively, are associated with serum levels of TGF-β [14]. Despite the controversy in the serum levels of TGF-β in coronary artery disease (CAD), TGF-β genotype has been shown to be a risk factor for genetic susceptibility to coronary atherosclerosis [15].

Several risk factors such as age, male gender, acute rejection, C-reactive protein (CRP), hyperhomocysteinaemia, hypertension, dyslipidemia, smoking and diabetes are defined as independent predictors of CVDs in RTR. However, conventional risk factors for CVDs are poor predictors of outcome in renal transplant population contrary to the case in the general population. In this study IL-10 promoter genes −1082 (G → A), −819 (C → T) −592 (C → A), TGF-β exon 1, codon 10 (T → C), codon 25 (G → C), TNF-α −308 (A → G) and −238 (G → A) genotypes, carotid intima media thickness (CIMT), left ventricular mass index (LVMI), 24-h ambulatory blood pressure and metabolic risk factors such as serum lipoproteins and homocysteine levels of RTR were determined to find out whether the genotypes of these cytokines contribute to cardiovascular complications of renal transplantation.

Patients and methods

Seventy-two renal transplant patients (26 cadaveric allograft, 46-living related allograft, 43 male, 29 female) were involved in this study. The characteristics of the study population are presented in Table 1. Patients who had serum creatinine >2.5 mg/dl, had a diagnosis of diabetes, impaired glucose tolerance, chronic infectious disease, CAD and other vascular disease were excluded in this study. All patients were receiving triple immune suppressive therapy with prednisolone, cyclosporin/tacrolimus and azathioprine/mycophenolate mofetil. The oral dose of cyclosporin and tacrolimus was based on the measurement of blood levels. The doses of azathioprine and mycophenolate mofetil were 1.5 and 30 mg/kg/day respectively. The patients had been using a maintenance dose of 5–10 mg daily at the time of

Table 1. Demographic findings and laboratory parameters of renal transplant patients.

Sex (male/female)	43/29
Age (years)	39.5 ± 11
Atiology of renal disease	
Chronic glomerulonephritis	41.6%
Vascular	15.2%
Chronic pyelonephritis	11.0%
Amyloidosis	4.1%
Adult polycystic kidney disease	2.7%
Miscellaneous	25.0%
Duration of dialysis months	25.5 ± 32.6
Duration of transplantation months	80.7 ± 56.2
Immunosupressive agent (no. of patients)	
Cyclosporin	62
Steroid	72
Azethiopirine	54
Mycofenolate mofetil	15
Tacrolimus	4
Body mass index (kg/m^2)	24.6 ± 4.1
Smoking	27.7%
Ambulatory systolic (mmHg)	125.8 ± 10.3
Ambulatory diastolic (mmHg)	80.9 ± 7.5
Antihypertensive no. of agents	1.47 ± 0.76
Glomerular filtration rate (ml/min)	81 ± 33
Haemoglobulin (g/l)	13.7 ± 1.9
Total cholesterol (mg/dl)	203 ± 45
LDL-C (mg/dl)	111 ± 36
HDL-C (mg/dl)	55 ± 16
Triglycerides (mg/dl)	170 ± 93
Lp (a) (mg/dl)	22.9 ± 31.2
Erythrocyte sedimentation rate (mm/h)	18.5 ± 15.1
C-reactive protein (mg/dl)	0.8 ± 2.2
Fibrinogen (mg/dl)	375 ± 88

LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; Lp (a), lipoprotein (a).

study. All patients have been previously diagnosed as hypertensive and except five (6.8%) all patients were receiving one or more antihypertensive medications (mean, 1.47 ± 0.76). Five of the patients were treated with only nonpharmacological treatment. The study was carried out in accordance with the Declaration of Helsinki (1989) and informed consent was obtained for all patients.

Ambulatory blood pressure

Twenty-four-hour ambulatory blood pressure monitoring was applied using a Spacelab (Redmond, WA, USA) oscillometric blood pressure monitor. Blood pressure was measured every 30 min from 02:00 to 02:00 PM. Measurements were only included if >85% of the readings were successful. Mean 24 h systolic blood pressure, diastolic blood pressure and mean arterial pressure were recorded in all patients.

Echocardiography

A single observer using a Toshiba SSH echocardiographic system with a 2.5-MHz transducer (Toshiba Inc., Tokyo, Japan) undertook m-mode, two-dimensional and Doppler echocardiographic examination. Left ventricular measurements were made at end diastole and end systole according to the recommendations of the American Society of Echocardiography. Left ventricular mass (LV mass) was calculated using the following equation based on the necroscopy validation studies [16]: LV mass $= 1.04 \times [(\text{LVIDd} + \text{IVSd} + \text{PWd})^3 - (\text{LVIDd})^3 - 13.6]$, where IVSd is interventricular septal thickness at end diastole, LVIDd is left ventricular internal dimension at end diastole, and PWd is posterior wall thickness at end diastole. LV mass (gram) is corrected for body surface area to give the LVMI (g/m^2).

Common carotid B-mode ultrasound

High-resolution B-mode ultrasound imaging of the common carotid arteries with scanning of the longitudinal axis until bifurcation and of the transversal axis was performed using an instrument generating a wide-band ultrasonic pulse with a middle frequency of 7.5 MHz (Siemens Inc., Erlangen, Germany). For each carotid artery, two longitudinal measurements were obtained by rotating (180° increments) the vessels along the axis. One experienced operator blindly examined all patients. CIMT is measured at 1 cm proximal to the bifurcation on each side as previously described [2].

Serum lipoproteins and inflammatory markers and glomerular filtration rate measurements

Blood samples were collected after an overnight fasting. Total cholesterol and triglycerides were quantified by commercial colorimetrical assay methods (GPO-PAP and CHOD-PAP; Boehringer-Mannheim, Mannheim, Germany). High-density lipoprotein-cholesterol (HDL-C) was quantified by the phosphotungustic acid precipitation method. Low-density lipoprotein-cholesterol (LDL-C) was calculated by Friedewald formula (LDL-C = CHO − TG/5 − HDL-C) where CHO is total cholesterol and TG is triglycerides. Lipoprotein (a) levels are quantified by rate nephelometer method (IMAGE; Backman, CA, USA). Plasma total homocysteine levels were quantified by the fluorescence polarisation immunoassay method (IMX System, Abbott, Germany). Plasma fibrinogen levels were determined by Diagnostica Stago system (Diagnostica Stago Inc., Paris, France) and erythrocyte sedimentation rate was measured by Sedisystem (Becton Dickinson, Paris, France) and CRP was

detected by rate nephelometry (IMAGE). Biochemical parameters (creatinine, BUN, glucose, electrolytes, albumin and complete blood count) were measured by means of a computerized autoanalyser (Hitachi 717; Boehringer Mannheim). Glomerular filtration rate (GFR) of patients was calculated with the Cockcroft–Gault equation.

Genotype

Venous blood (10 ml) was collected in tubes containing 50 mmol disodium EDTA and genomic DNA was isolated with a DNA extraction kit. All patients were genotyped for IL-10, TGF-β and TNF-α with polymerase chain reaction (PCR) sequence-specific assay (cyclerplate system cytokine). The assay consists of ready-to-use primer mixes prepipetted in thin-walled wells. It contains eight IL-10-specific primers, eight TGF-β-specific primers and four TNF-α-specific primers. The thermocycling procedure consists of initial denaturation at 94 for 2 min, 10 cycles of denaturation (94 for 10 s) 10 cycles of annealing and extension (65 for 1 min), 20 cycles of denaturation (94 for 10 s), annealing (61 for 50 s) and extension (72 for 30 s). The PCR products were analysed by 1% agorose gel electrophoresis and visualized with ethidium bromide. The expected size of specific amplification of 20 different specific primers was evaluated in accordance with the manufacturer's instruction and IL-10 promotor −1082 (G → A), −819 (C → T) and −592 (C → A), TGF-β exon 1, codon 10 (T → C), codon 25 (G → C), TNF-α −308 (A → G) and −238 (G → A) genotypes were determined.

Statistical analysis

Statistical analysis was performed using SPSS, version 10.0. Data are presented as mean ± SD. Kolmogorov–Smirnov test was used to evaluate normality. Hardy–Weinberg equilibrium was assessed by chi-square analysis. One-way analysis of variance (ANOVA) was used to compare quantitative parameters across genotypes. Student's *t*-test was used for grouped comparison of continuous variables between high and low producers.

Results

Allele frequency and genotype distribution of IL-10 [−1082 (G → A), −819 (C → T), −592 (C → A)], TGF-β1 [codon 10 (T → C), codon 25 (G → C)] and TNF-α [−308 (A → G), −238 (G → A)] are presented in Table 2. No difference was observed with regard to age, sex, body mass index, duration of haemodialysis and transplantation, habitual smoking, number of antihypertensive drugs, 24-h ambulatory blood pressure levels, GFR, haemoglobin levels, serum lipoproteins, plasma homocysteine level, erythrocyte sedimentation rate, serum CRP and serum fibrinogen in patients with TNF-α −308 (AA, AG, GG), TNF-α −238 (AA, AG, GG), IL-10 −1082 (AA, AG, GG), IL-10 −819 (CT, TT, CC), IL-10 −592 (AA, AC, CC) and TGF-β1 codon 10 (CT, TT, CC), TGF-β1 codon 25 (CC, CG, GG) genotypes.

Mean LVMI of the total patients was 110.5 ± 42.6 g/m^2 and mean right and left carotis intima thickness of the renal transplant patients was 7.0 ± 1.7 and

	Allele	Genotype	Phenotype/cytokine production
IL-1082	A, 51.3%	GG (*n* = 22), 30.6%	High GG (*n* = 22), 30.6%
	G, 48.7%	AA (*n* = 24), 33.3%	Low AA + GA (*n* = 50), 69.4%
		GA (*n* = 26), 36.1%	
IL-819	C, 64.6%	TT (*n* = 16), 22.2%	High CC (*n* = 37), 51.4%
	T, 35.4%	CC (*n* = 37), 51.4%	Low TT + CT (*n* = 35), 48.6%
		CT (*n* = 19), 26.4%	
IL-10 592	A, 43.1%	AA (*n* = 23), 31.9%	High CC (*n* = 33), 45.8%
	C, 56.9%	CC (*n* = 33), 45.8%	Low AA + AC (*n* = 39), 54.2%
		AC (*n* = 16), 22.3%	
TNF-α 308	A, 12.5%	AA (*n* = 2), 2.8%	High AA + GA (*n* = 16), 22.2%
	G, 87.5%	GG (*n* = 56), 77.8%	Low GG (*n* = 56), 77.8%
		GA (*n* = 14), 19.4%	
TNF-α 238	A, 5.6%	AA (*n* = 2), 2.8%	High AA + GA (*n* = 6), 8.4%
	G, 94.4%	GG (*n* = 66), 91.6%	Low GG (*n* = 66), 91.6%
		GA (*n* = 4), 5.6%	
TGF-β 25	C, 12.5%	CC (*n* = 1), 1.4%	High GG (*n* = 55), 76.4%
	G, 87.5%	GG (*n* = 55), 76.4%	Low CC + GC (*n* = 17), 23.6%
		GC (*n* = 16), 22.2%	
TGF-β 10	C, 45.9%	CC (*n* = 10), 14.0%	High TT + TC (*n* = 62), 86%
	T, 54.1%	TT (*n* = 16), 22.2%	Low CC (*n* = 10), 14%
		CT (*n* = 46), 63.8%	

Table 2. Allele frequency and genotype/phenotype distribution of interleukin-10 (IL-10) −1082 (G → A), −819 (C → T), −592 (C → A), transforming growth factor beta 1 (TGF-β1) codon 10 (T → C), codon 25 (G → C), tumour necrosis factor alfa (TNF-α) −308 (A → G) and −238 (G → A) polymorphisms in renal transplant patients.

7.2 ± 1.8 mm respectively. LVMI of high-producer patients and low-producer patients was similar in accordance with TNF-α −308 (AA + AG versus GG), TNF-α −238 (AA + AG versus GG), IL-10 −1082 (AA + AG versus GG), IL-10 −819 (CT + TT versus CC), IL-10 −592 (AA + AC versus CC) and TGF-β1 codon 10 (CT + TT versus CC), TGF-β1 codon 25 (CC + CG versus GG) genotypes (Table 3). CIMT of low producers for anti-inflammatory cytokine IL-10 −1082 was greater than that of high producers, but the difference did not reach statistical significance ($P > 0.05$). CIMT of renal transplant patients was similar for IL-10 −819, IL-10 −592, TNF-α −308 and TNF-α −238 gene polymorphisms (Table 3). The right and left IMTs of carotid artery were significantly greater in high producers for TGF-β1 codon 10 genotype (TT + TC) compared with low producers (CC) (7.2 ± 1.7 mm vs. 5.9 ± 1.7 mm, $P = 0.023$; 7.5 ± 1.8 mm vs. 6.1 ± 1.6 mm, $P = 0.024$) (Fig. 1). Right and left CIMT were higher in the TT genotype than in the CT and CC genotypes of TGF-β1 codon 10 T → C gene polymorphism (RCIMT, 7.7 ± 2.2 mm vs. 7.0 ± 1.4 mm vs. 5.9 ± 1.4 mm, $P = 0.025$; LCIMT, 8.5 ± 2.5 mm vs. 7.0 ± 1.3 mm vs. 6.1 ± 1.2 mm, $P = 0.002$) Compared with low producers (GC + CC), right and left IMT of carotid artery was greater in high producers for TGF-β codon 25 (GG) (7.2 ± 1.7 mm vs. 6.3 ± 1.2 mm and 7.4 ± 1.9 mm vs. 6.6 ± 1.7 mm respectively) but this difference did not reach statistical significance ($P = 0.14$, $P = 0.10$ respectively).

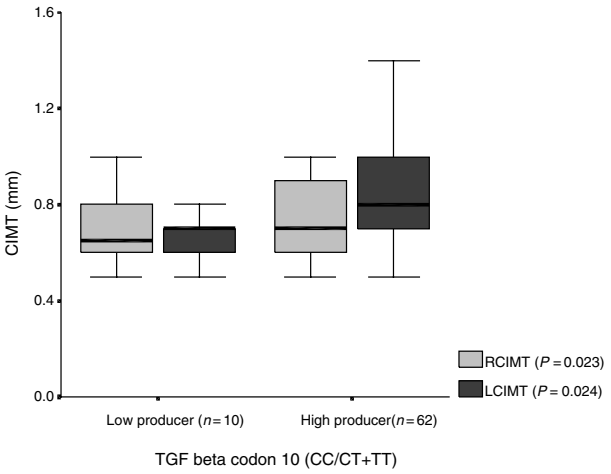

Figure 1 Right and left carotid artery intima media thickness (CIMT) of high producer patients (TT + TC) and low producer patients (CC) according to transforming growth factor beta 1 (TGF-β1) codon 10 (T → C) polymorphism.

Lipoprotein (a) level of the TT genotype (35.5 ± 22.5 mg/dl) was higher than the CC (4.1 ± 2.8 mg/dl) and CT (20.4 ± 11.2 mg/dl) genotypes of TGF-β1 codon 10 (T → C) gene polymorphism

Table 3. Left ventricular mass index (LVMI) and carotid artery intima media thickness (CIMT) according to genotype distribution.

Genotype	LVMI (g/m^2)	Right CIMT (mm)	Left CIMT (mm)
IL-10 1082			
High producer (GG) ($n = 22$)	107 ± 43	6.7 ± 1.4	7.0 ± 0.1
Low producer (AA + GA) ($n = 50$)	116 ± 40	7.5 ± 2.1	7.7 ± 2.5
IL-10 819			
High producer (CC) ($n = 37$)	114 ± 49	6.8 ± 1.2	7.0 ± 1.8
Low producer (CT + TT) ($n = 35$)	106 ± 33	7.1 ± 2.0	7.5 ± 1.7
IL-10 592			
High producer (CC) ($n = 33$)	116 ± 38	6.6 ± 1.3	7.2 ± 1.7
Low producer(AA + CA) ($n = 39$)	105 ± 44	7.2 ± 1.9	7.2 ± 1.8
TNF-α 308			
High producer(AA + AG) ($n = 16$)	107 ± 36	6.6 ± 1.4	7.1 ± 1.5
Low producer (GG) ($n = 56$)	111 ± 44	7.0 ± 1.7	7.3 ± 1.8
TNF-α 238			
High producer (AA + AG) ($n = 6$)	109 ± 37	6.7 ± 1.5	6.6 ± 1.5
Low producer (GG) ($n = 66$)	110 ± 43	7.0 ± 1.7	7.3 ± 1.8
TGF-β1 25			
High producer (GG) ($n = 55$)	109 ± 35	7.2 ± 1.7	7.4 ± 1.9
Low producer (GC + CC) ($n = 17$)	112 ± 59	6.3 ± 1.2	6.6 ± 1.7
TGF- β1 10			
High producer (TT + TC) ($n = 62$)	109 ± 42	7.2 ± 1.7	7.5 ± 1.8
Low producer (CC) ($n = 10$)	117 ± 40	5.9 ± 1.1**	6.1 ± 1.2*

Values are expressed as mean ± SD.
IL-10, interleukin-10; TNF-α, tumour necrosis factor alpha; TGF-β1, transforming growth factor beta 1.
*P, = 0.024; **P, = 0.023.

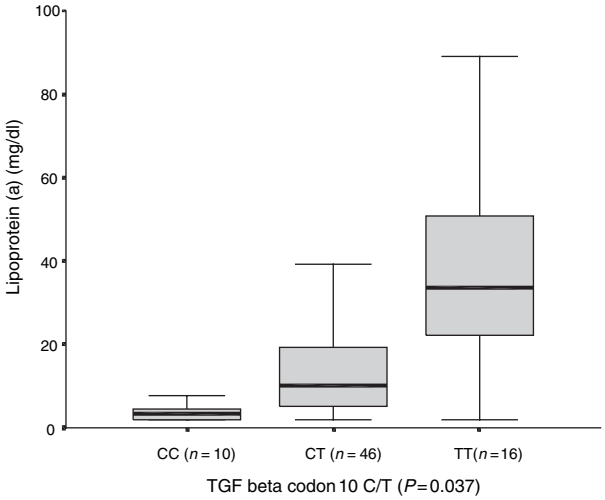

Figure 2 Lipoprotein (a) levels of patients with TT, TC and CC genotypes of transforming growth factor beta 1 (TGF-β1) codon 10 (T → C) polymorphism.

Table 4. Demographic and laboratory parameters of patients according to transforming growth factor beta 1 (TGF-β) codon 10 polymorphism in renal transplant patients.

Parameter	High producer	Low producer
Genotype	TT + TC	CC
Age (years)	40.5 ± 11.3	38.2 ± 6.0
Sex (female/male)	24/38	5/5
Duration of dialysis months	25.2 ± 32.8	27.7 ± 33.1
Duration of transplantation months	68.4 ± 42.2	66.8 ± 32.4
Body mass index (kg/m^2)	24.3 ± 3.4	26.5 ± 6.9
Smoking (patient %)	29%	40%
Ambulatory systolic (mmHg)	126 ± 10	124 ± 12
Ambulatory diastolic (mmHg)	81 ± 7	80 ± 8
Antihypertensive number	1.42 ± 0.76	1.44 ± 0.72
Antihypertensive agent (%)	93	90
ACE inhibitor	27.4	22.2
Angiotensin II receptor blocker	34.4	33.4
Ca channel blocker	56.8	66.6
Beta blocker	24.1	22.2
Glomerular filtration rate (ml/min)	78.9 ± 31.9	97.3 ± 37.9
Haemoglobulin (g/dl)	13.7 ± 1.9	13.3 ± 1,6
Total cholesterol (mg/dl)	205 ± 47	193 ± 30
LDL-C (mg/dl)	112 ± 38	108 ± 25
HDL-C (mg/dl)	55 ± 17	55 ± 12
Triglyceride (mg/dl)	175 ± 99	138 ± 36
Statin (%)	13.7	10
Homocysteine (ng/ml)	21.8 ± 10.1	32 ± 29.4
Lp (a) (mg/dl)	24.5 ± 22.6	4.1 ± 2.8
Erythrocyte sedimentation rate (mm/h)	18.0 ± 14.8	21.3 ± 17.4
C-reactive protein (mg/dl)	0.7 ± 2.2	1.1 ± 2.7
Fibrinogen (mg/dl)	375 ± 79	378 ± 135

Values are expressed as mean ± SD.
ACE, angiotensin-converting enzyme; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; Lp (a), lipoprotein (a).

(Fig. 2, $P = 0.037$ and $P = 0.040$ respectively). Age, sex, body mass index, duration of haemodialysis and transplantation, habitual smoking, number of antihypertensive drugs, 24-h ambulatory blood pressure levels, GFR, haemoglobin levels, serum lipoproteins, plasma homocysteine level, erythrocyte sedimentation rate, serum CRP and fibrinogen were similar in high- and low-producer groups of TGF-β1codon 10 (Table 4).

The CIMT and LVMI showed no significant correlation with age, sex, body mass index, duration of haemodialysis and transplantation, habitual smoking, number of antihypertensive drugs, 24-h ambulatory blood pressure levels, GFR, haemoglobin levels, serum lipoproteins, lipoprotein (a), plasma homocysteine level, erythrocyte sedimentation rate, serum CRP and fibrinogen.

Discussion

Recently several studies have focused on the role of cytokines in generating CVD. In this study we analysed the possible association of the functional polymorphisms of TNF-α, IL-10, TGF-β genes with atherosclerosis and LVMI in RTR. Hoffman *et al.* [17] studied the influence of ethnicity on cytokine gene polymorphism in racially diverse end-stage renal failure patients on transplantation waiting list and our cytokine gene polymorphism distributions are consistent with white population of this study. To the best of our knowledge this is the first study reporting the association of cytokine genotypes and cardiovascular disease after renal transplantation.

Tumour necrosis factor-α has been implicated in the pathogenesis of cardiovascular disease [5]. Secretion of TNF-α from leucocytes is increased in patients with stable or unstable angina pectoris in comparison with healthy controls [18]. Additionally, elevated local and circulating TNF-α levels have been shown in patients with myocardial infarction [19]. However, association between polymorphisms in the TNF-α promoter region and cardiovascular disease is controversial [9,20,21]. TNF-α allele −308 (G → A) is associated with higher transcriptional levels and has been reported to be increased in frequency in patients with unstable angina pectoris [20]. Moreover, the frequency of coronary thrombosis and stenosis was similar in different genotype status in locus TNF-α at position −308 in the autopsy series in Caucasian subjects [21]. Despite the elevated serum levels [18], no association between the TNF-α genotype and congestive heart failure has been reported [22]. No significant differences were observed in CIMT and LVMI in accordance with the TNF-α genotype in RTR. Consistent with our findings, the development of cardiac transplant-related vasculopathy in heart transplant recipients is not associated with the TNF-α gene polymorphism [23]. From

the foam cells to rupture of plaque, inflammation plays an important role in every stage of atherosclerosis. However, this inflammation is continuous but weak in nature and augmented after disruption and rupture of atherosclerotic plaques in acute cardiovascular events. So inflammatory process in the carotid vascular wall in our patients is probably not associated with explosive inflammatory cytokine production and genetic difference in the productive capacity of cytokine may not be evident in asymptomatic early atherosclerosis.

Interleukin-10 is a potent anti-inflammatory cytokine and probably because of local production by the macrophages is found within the atheromatous plaque [11]. Clinical studies of the role of plasma IL-10 levels with atherosclerotic disease are inconclusive. Elevated plasma IL-10 levels in 50 patients with stable, compared with 45 patients with unstable, coronary syndrome have been reported [24]. Despite positive correlation between serum TNF-α concentration, Mizia-Stec *et al.* [18] could not establish any relationship between serum IL-10 concentration and LVMI of patients with stable angina pectoris. The IL-10 gene has at least three polymorphisms that are related to cytokine expression. The (G $\rightarrow$ A) base exchange at position -1082 in the promoter of the gene leads to about 30% less IL-10 protein upon a definite stimulus [12]. Similarly, but to a lesser extent, exchange in the bases of -819 (C $\rightarrow$ T) and -592 (C $\rightarrow$ A) also result in lower production of the cytokine upon stimulus. Two large studies investigating patients with angiographically proven CAD or myocardial infarction did not detect any relationship between a specific combination of IL-10 genotypes and CAD [25, 26]. In contrast to these studies involving patients with normal renal function, IL-10 -1082 A allele is predictive for a higher cardiovascular morbidity compared with the -1082 G genotype in dialysis patients [27]. Although it is not statistically significant, high producers for IL-10 -1082, had lower IMT of carotid artery compared with low producers in RTR in the present study. These findings suggest that the IL-10 gene polymorphism might influence the risk for cardiovascular events in renal patients. However, the differences observed in patients who had undergone haemodialysis and renal transplantation might be due to cytokine production. Given the increased production of inflammatory anti-inflammatory cytokines in haemodialysis, the inflammatory component of atherosclerosis might be strongly enhanced in end-stage renal failure patients compared with renal transplant patients.

Transforming growth factor-β inhibits the proliferation and migration of vascular smooth muscle, induces the expression of collagen genes and stimulates the accumulation of collagen within the vascular wall [5]. TGF-β1 is synthesized as pro TGF-β1 which is subsequently cleaved between the C-terminal and the larger N-terminal peptides (known as latency-associated peptides) remain noncovalently associated with the C-terminal peptides thus forming latent forms of the TGF-β1 protein [28]. In patients with severe CAD the serum concentration of active TGF-β was one-fifth of that in individuals with normal coronary arteries [29]. Similarly, serum levels of the active TGF-β1 were severely depressed in nonrenal patients with advanced atherosclerosis [30]. TGF-β1 is significantly reduced in haemodialysis patients, in particular in those with severe CVD [31]. In contrast to these findings, Grainger *et al.* [30] found that the serum concentration of active and latent TGF-β did not differ between controls and patients with CAD. TGF-β1 concentration may be a causal factor or compensatory response to CAD as the serum level of this cytokine may be influenced by factors such as CAD status and treatment and concomitant disorders such as diabetes mellitus and hypercholesterolaemia.

Recent evidence indicates that there is a substantial genetic component controlling total (acid activatable latent form of TGF-β plus active TGF-β) and active TGF-β concentration in the circulation. Two polymorphisms (T10 $\rightarrow$ C, G25 $\rightarrow$ C) in the coding region of the gene are located at potentially important positions for activation of the TGF-β1 protein resulting in changes in the protein sequence Leu/Pro and Arg/Pro [14]. By influencing intracellular signalling or export efficiency of preprotein, the Leu/Pro and Arg/Pro polymorphisms may affect the gene expression of this cytokine [14]. The correlation between serum TGF-β levels and gene polymorphisms has been studied in various patient groups. The serum concentration of TGF-β was higher in individuals with the CC genotype at codon 10 than in those with the TT or TC genotype in myocardial infarction patients [32]. However, Awad *et al.* [33] showed that the T allele is linked to higher production of TGF-β1. These findings suggest that the level of TGF-β in the peripheral blood is not uniform and may not reflect tissue levels of this cytokine. Gene expression of a cytokine is the best way of evaluating the intrinsic production capacity. In renal transplant patients codon 10 TT and codon 25 GG genotypes have the highest gene expression compared with others and the effect of these alleles on gene expression is amplified in patients undergoing cyclosporin treatment [34]. Despite the correlation between TGF-β1 polymorphism and gene expression, no difference was observed in serum levels of the cytokine in these patients.

The role of TGF-β1 gene polymorphisms as a genetic risk factor for predisposition to atherosclerosis in the general population is controversial. Increased frequency of T allele at codon 10 in TGF-β1 gene has been repor-

ted in Japanese male patients with myocardial infarction [32]. The Pro 25 allele is associated with an increased risk of myocardial infarction but not with the angiography findings of patients with CAD in the ECTIM study [15]. On the other hand, Syrris *et al.* [35] failed to detect an association of the prevalence of CAD with either the (T10 → C) or the (G25 → C) polymorphism of the TGF-β1 gene. The present study demonstrated that T alelle of the (T10 → C) polymorphism, which is associated with increased TGF-β1 gene expression, is a risk factor for genetic susceptibility to early atherosclerosis in RTR. These findings suggest that compared with the general population, the influence of genetic variation of the TGF-β1 production on atherosclerosis may be more pronounced in RTR and may be present even at early stages of atherosclerosis. The difference between the general population and RTR is probably related to immunosuppressive treatment. Cyclosporin treatment increases both serum and tissue levels of TGF-β1 and moreover, cyclosporin itself may amplify the differences in gene expression of TGF-β1 in relation to genotype in RTR [34]. In our study, levels of lipoprotein (a) were higher in patients with the T → T polymorphism at TGF-β1 gene codon 10. The latent, inactive form of TGF-β1 is activated proteolytically by plasmin and lipoprotein (a) blocks this activation by competitively inhibiting he plasminogen activator [36]. Lipoprotein (a) therefore promotes vascular smooth muscle cell proliferation by relieving the autocrine inhibition of active TGF-β1. So elevated levels of lipoprotein (a) in T → T genotype might be an association as these patients are more prone to atherosclerosis or a reactive increase to inhibit vascular effects of TGF-β1 in high producers of this cytokine.

In conclusion, besides the well-known factors, TGF-β1 gene polymorphisms might play a determinant role in the formation of atherosclerotic lesions in RTR. Lack of association between IL-10 genotypes, TNF-α genotypes and carotid atherosclerosis and LVM in this study should not be interpreted as data negating the potential role of cytokines in the development of renal transplant-related CVD. Further prospective controlled studies are necessary to evaluate the role of cytokine gene polymorphisms in CVD in RTR.

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