Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Renal transplant recipients with coronary artery disease exhibit impairment in fibrinolysis and structural changes in carotid arteries

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Keywords
coronary artery disease, fibrinolysis, hemostasis, intima-media thickness, kidney transplantation.

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Received: 2 October 2003
Revised: 2 September 2004
Accepted: 23 September 2004

doi:10.1111/j.1432-2277.2004.00027.x

Summary

Cardiovascular disease (CVD) is the main cause of mortality and morbidity among kidney transplant recipients (Tx). Intima-media thickness (IMT) of the common carotid artery is related to CVD. Hemostatic disturbances may contribute to the CVD pathogenesis in Tx. The aim of the study was to evaluate some hemostatic factors in Tx with and without coronary artery disease (CAD) and their correlations with IMT. Patients with CAD were significantly older, with thicker IMT, lower plasmin–antiplasmin complexes (PAP), higher fibrinogen, cholesterol, triglycerides, Thrombin-activatable fibrinolysis inhibitor (TAFI) antigen and activity, prolonged euglobulin clot lysis time when compared to those without CAD. Kidney transplant recipients have higher mean blood pressure, serum lipids, fibrinogen, TAFI antigen, TAFI activity, markers of coagulation and fibrinolysis, thicker IMT and lower PAP relative to healthy volunteers. In univariate analysis, IMT correlated significantly with age, time on dialysis prior to transplantation, PAP, fibrinogen, hematocrit, body mass index (BMI). Multiple regression analysis showed that only age, hematocrit, PAP, and time on dialysis prior to transplantation were positive independent predictors of IMT. Hypofibrinolysis may contribute to the arterial remodeling in Tx. Dialysis therapy before transplantation makes detrimental changes in arterial vasculature.

Introduction

The incidence of cardiovascular disease (CVD) in kidney transplant recipients appears to be increased three- to fourfold over that observed in age-matched healthy subjects [1]. CVD is the most common cause of morbidity and mortality in the renal transplant population. Independent risk factors for CVD in kidney transplant recipients include age, pretransplant diabetes, male sex, acute rejection episodes, pretransplant peripheral, cerebrovascular or ischemic heart disease [2]. Additional risk factors include disturbances in hemostasis, immunosuppressive therapy, time on dialysis prior transplantation, and obesity [3]. Hypercoagulable state, endothelial cell injury and impaired fibrinolysis were described in kidney transplant recipients [4–6]. Recently, we have reported an enhanced thrombin generation and increased thrombin-activatable fibrinolysis inhibitor (TAFI) in kidney transplant recipients with dyslipidemia [7]. Moreover, high TAFI is associated with increased risk of acute coronary artery disease (CAD) [8]. Markers of endothelial cell injury are also predictors of coronary events [9].

Intima-media thickness (IMT) of the carotid artery could be a marker of early atherosclerotic changes [10]. In addition, carotid IMT may be a useful, noninvasive method for assessment of cardiovascular risk because of its correlation with presence of CVD [11–13].

In this study, we addressed the question whether kidney transplant recipients with CAD differ from those without CAD in regard to some hemostatic parameters and IMT. We also investigated correlations between IMT and parameters studied in these two groups.

Patients and methods

The studies were performed on 48 renal allograft recipients (20 women, 28 men, age range: 26–73 years). The immunosuppressive regimen consisted of CyA (3.41 ± 1.2 mg/kg body weight, blood through CyA levels – 100–250 ng/ml), prednisone (7.5–10 mg daily), and azathioprine (100–150 mg daily). All of them maintained sufficient and stable graft function without clinical signs of rejection, inflammation (C-reactive protein within normal range) and liver dysfunction (prothrombin time, alanine aminotransferase within normal range). CAD was diagnosed in 25 patients (52%) on the basis of having the history of myocardial infarction (MI), typical changes in coronary angiograms, typical ischemic changes in electrocardiogram, or presence of typical angina pectoris. Arterial hypertension was diagnosed in 41 patients (85%). Healthy volunteers ($n = 24$) were recruited from hospital staff and their families. All the patients were informed about the aim of the study and gave their consent; Local Ethical Committee also approved the study. All the subjects underwent measurements of carotid artery IMT by high-resolution real-time B mode ultrasonography with 7,5 MHz linear transducer (SSH 140A Toshiba, Japan) as described previously [14]. Blood was drawn without stasis ratio in the morning. Total cholesterol, triglycerides, euglobulin clot lysis time (ECLT), fibrinogen, albumin concentration were measured by standard laboratory methods. Cyclosporine levels were assessed using AxSYM system with polarized fluorescence. In citrated plasma, we evaluated thrombomodulin (TM; American Diagnostica, Inc., Greenwich, CT, USA), prothrombin fragments F1 + 2 (Dade Behring, Marburg, Germany), thrombin–antithrombin complexes (TAT; Dade Behring), plasmin–antiplasmin complexes (PAP; Dade Behring), TAFI concentration (Affinity Biologicals, Inc., Hamilton, ON, Canada), and activity (American Diagnostica, Inc., Greenwich, CT, USA) with commercially available kits. To make ECLT independent on fibrinogen concentration, we calculated fibrinolytic activity index (FAI = fibrinogen divided by ECLT). Data were expressed as mean ± SD. Comparisons between groups were done by ANOVA for normally distributed variables and Kruskall–Wallis ANOVA for non-normal distribution of variables. Correlation between IMT and other variables were evaluated by Pearson's or Spearman's test as appropriate. Values of $P < 0.05$ were taken as statistically significant.

Results

Patients with CAD were older, with thicker IMT, higher cholesterol, low-density lipoprotein (LDL), fibrinogen, lower platelet count, serum albumin, total protein, PAP, and prolonged ECLT when compared to patients without the CAD and the healthy volunteers. Patients without CAD had higher mean arterial blood pressure, total cholesterol, LDL, triglycerides, erythrocyte count, hematocrit, TAFI concentration and activity, TM, F1 + 2, and TAT relative to the control group. FAI was similar in all groups studied (1.19 ± 0.4 in the healthy volunteers, 1.21 ± 0.8 in Tx with CAD, 1.15 ± 0.6 in Tx without CAD).

In univariate analysis, IMT correlated significantly with age ($r = 0.60$; $P < 0.001$), time on dialysis prior to transplantation ($r = 0.57$; $P < 0.001$), PAP ($r = -0.56$, $P < 0.001$), fibrinogen ($r = 0.51$; $P < 0.05$), hematocrit ($r = 0.46$, $P < 0.01$), BMI ($r = 0.35$, $P < 0.05$). IMT correlated positively at the level of statistical significance with F1 + 2 ($r = 0.29$, $P = 0.06$). Multiple regression analysis showed that only age ($\beta = 0.486$), hematocrit ($\beta = 0.329$), PAP ($\beta = 0.26$), and time on dialysis prior to transplantation ($\beta = 0.114$) were positive independent predictors of IMT, accounting for 58% of its variability. In the healthy volunteers, IMT correlated significantly only with age ($r = 0.63$, $P < 0.01$) and BMI ($r = 0.64$, $P < 0.01$).

Discussion

In the general population, there is a relation between carotid IMT and traditional cardiovascular risk factors like arterial hypertension, diabetes mellitus, smoking, or hyperlipemia [15]. Kidney transplant recipients with CAD had thicker IMT when compared to those without CAD and to the healthy volunteers. It should be stressed that carotid IMT was positively related to age. Correlations between carotid IMT and age were reported previously [10]. They reflect a progression of athero/arteriosclerotic changes following natural aging process. Correlation between IMT and time on hemodialyses prior to transplantation indicate the role of dialysis treatment on the progression of arterial remodeling. It is supported by the fact that post-transplant CAD is closely linked to pre-transplant CAD [1,2]. Fibrinogen is an independent risk factor for CAD [16] and high cardiovascular mortality [17]. In patients with CAD, fibrinogen was higher relative to subjects without CAD. Fibrinogen together with other markers of inflammation, e.g. C-reactive protein, endothelial cell glycoproteins, fibrinolysis inhibitors, and cytokines are predictors of cardiovascular death in patients with kidney diseases [18]. Previously, we reported that

Table 1. Comparison of some biochemical and hemostatic parameters in kidney transplant recipients (Tx) with and without coronary artery disease (CAD) and in the healthy volunteers.

	Healthy volunteers ($n = 24$)	Tx with CAD ($n = 25$)	Tx without CAD ($n = 23$)
Age (years)	42.5 ± 6.9	51.0 ± 9.5†	36.1 ± 6.1***
BMI (kg/m^2)	24.4 ± 3.2	25.9 ± 3.7	23.7 ± 3.4
Mean arterial pressure (MAP) (mmHg)	95.1 ± 3.1	108.2 ± 5.6†††	105.1 ± 4.9††
Creatinine clearance (ml/min)	107.5 ± 8.6	49.9 ± 3.9	58.9 ± 24.1
Time after Tx (months)	Not applicable	48.9 ± 35.1	40.1 ± 23.8
CsA concentration (ng/ml)	Not applicable	142.9 ± 46.3	126.8 ± 50.0
Total cholesterol (mg/dl)	179.9 ± 22.1	242.3 ± 31.8†††	216.7 ± 41.8*††
LDL cholesterol (mg/dl)	109.7 ± 22.5	140.2 ± 42.0†††	133.5 ± 32.3†††
HDL cholesterol (mg/dl)	62.3 ± 12.8	67.2 ± 22.7	58.9 ± 17.1
Triglycerides (mg/dl)	89.5 ± 13.2	182.2 ± 58.5†††	143.6 ± 43.9†††*
Fibrinogen (mg/dl)	239.8 ± 39.7	321.8 ± 72.0†††	265.0 ± 60.8***
Hematocrit (%)	45.8 ± 2.4	42.7 ± 7.3†	39.1 ± 7.9††
Erythrocyte count (×10^{12}/l)	4.79 ± 1.89	4.49 ± 0.91†	4.38 ± 1.25††
Leukocyte count (×10^3/l)	6.42 ± 1.64	6.25 ± 1.59	5.46 ± 1.27*
Platelet count (×10^9/l)	224.3 ± 48.5	154.7 ± 45.9†	204.7 ± 58.3*
Albumin (g/dl)	4.46 ± 0.32	4.03 ± 0.43†	4.29 ± 0.42*
Total protein (g/dl)	7.11 ± 0.52	6.66 ± 0.54†	7.06 ± 0.63*
ECLT (min)	201.8 ± 89.5	265.4 ± 90.8†	230.0 ± 61.2*
PT (s)	13.2 ± 1.3	13.6 ± 1.4	13.5 ± 1.6
APTT (s)	35.8 ± 5.9	37.2 ± 5.6	34.2 ± 7.6
IMT (mm)	0.61 ± 0.12	0.74 ± 0.12†††	0.63 ± 0.08**
TAFI concentration (% of standard plasma)	114.2 ± 48.5	293.7 ± 124.4†††	238.4 ± 102.0*†††
TAFI activity (μg/ml)	3.21 ± 0.83	8.51 ± 2.28†††	6.80 ± 2.97*††
Thrombomodulin (ng/ml)	3.37 ± 1.64	7.06 ± 4.29†††	6.93 ± 3.56†††
F1 + 2 (nm)	1.14 ± 0.71	2.43 ± 1.84††	1.85 ± 0.87†
TAT (μg/l)	1.5 (0–3)	15 (2–134)†††	10 (1–78)†††
PAP (μg/l)	584 ± 112	359.0 ± 146.7†††	668.5 ± 481.1***

BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ECLT, euglobulin clot lysis time; APTT, activated partial thromboplastin time; IMT, intima-media thickness; TAFI, thrombin-activatable fibrinolysis inhibitor; TAT, thrombin–antithrombin complex; PAP, plasmin–antiplasmin complex; CVD, cardiovascular disease.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, CVD (+) versus CVD (–).

†$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ versus control group.

IMT correlated positively with hemoglobin and hematocrit in kidney transplant recipients [14]. Multiple regression analysis revealed that IMT was independently related to age, hemoglobin, hematocrit, and fibrinogen. We postulated a possible role of these rheologic factors in the progression of arterial remodeling in kidney transplant recipients [14] (Table 1).

Hemostatic system plays an important role in the pathogenesis of atherosclerotic vascular disease [19]. In our study, patients with CAD had a significantly elevated TAFI concentration and activity, lower PAP complexes, prolonged ECLT indicating an impaired fibrinolysis, when compared to patients without CAD. In both groups of kidney transplant recipients, significantly elevated markers of ongoing coagulation – TAT and F1 + 2 were found. However, at first sight, it may seem confounding that kidney transplant recipients without CAD and the healthy volunteers did not differ with regard to PAP. When we take into account the hypercoagulability as reflected by elevated TAT and F1 + 2, we concluded that this lack of difference in PAP between healthy volunteers and kidney transplant recipients without CAD was another argument for hypofibrinolysis in this population. Moreover, FAI was similar in all three groups studied despite significantly prolonged ECLT in kidney transplant recipients with CAD. It suggests that prolongation of ECLT mainly depends upon fibrinogen. In addition, a negative correlation between PAP and IMT in kidney transplant recipients strengthens the role of hypofibrinolysis in the progression of atherosclerosis. Hypofibrinolysis is attributed to the use of immunosuppressive drugs [steroids and cyclosporin A (CsA)], but it was more pronounced in subjects with CAD. These two subgroups have similar CsA dose and concentration and dose of prednisone. In kidney transplant recipients, particularly with CAD, high TAFI level and activity were found. Elevated TAFI was also reported in patients with unstable angina pectoris [20] and in patients undergoing coronary artery bypass

grafting (CABG) [21]. In addition, elevated TAFI (above 126%) was associated with an almost fourfold higher risk of CAD [8]. High TAFI in hypercholesterolemic kidney transplant recipients may contribute to hypofibrinolysis and to the progression of atherosclerosis in these patients.

Hypofibrinolysis and elevated fibrinogen observed in kidney transplant recipients with CAD may contribute to the arterial remodeling in this population. It also seems conceivable that IMT reflects the risk for CAD in transplanted patients. Dialysis therapy before transplantation makes detrimental changes in arterial vasculature.

Acknowledgement

The study was supported by the grant from KBN (Polish Committee for Research) 6 POB 117 21. S. Brzosko is a recipient of scholarship from Polish Research Foundation.

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