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Original Paper

Relationship between Plasma Pentraxin-3, Neutrophil-to-Lymphocyte Ratio, and Atherosclerosis in Renal Transplant Patients

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Key Words
Pentraxin-3 · Neutrophil-to-lymphocyte ratio · High-sensitivity C-reactive protein · Carotid intima-media thickness · Renal transplantation

Abstract
Background/Aims: Atherosclerosis and inflammation are the most important risk factors in the pathogenesis of cardiovascular diseases (CVD) in patients with end-stage renal disease (ESRD). Pentraxin-3 (PTX-3) was shown to predict inflammation and atherosclerosis in ESRD patients. However, the role of renal transplantation (Rtx) in terms of atherogenesis is still unclear. We aimed to investigate the relationship between PTX-3, neutrophil-to-lymphocyte ratio (NLR), and carotid intima-media thickness (CIMT) in Rtx patients and healthy controls. ***Methods:*** This was a cross-sectional study involving 29 Rtx patients (12 females, 40.1 ± 11.9 years) without overt CVD and 19 healthy subjects (9 females, 36.9 ± 8.9 years), testing the relationship between CIMT, assessed by ultrasonography, and selected biomarkers. ***Results:*** CIMT, PTX-3, and high-sensitivity C-reactive protein (hs-CRP) levels of Rtx patients were found to be significantly higher compared to healthy subjects. CIMT was positively correlated with age, creatinine, uric acid, triglyceride, PTX-3, hs-CRP, and NLR, and negatively correlated with estimated glomerular filtration rate in all participants. In Rtx patients, CIMT was positively correlated with age, BMI, serum phosphorus, low-density lipoprotein, and hs-CRP. The multivariate analysis revealed that hs-CRP was found to be an independent variable of CIMT in Rtx patients. ***Conclusion:*** Our data showed that inflammation and atherosclerosis persist in Rtx patients. Serum hs-CRP might be a useful marker to assess these parameters in this population.

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Introduction

Atherosclerosis, endothelial dysfunction, and coronary artery calcification are the most commonly encountered risk factors in the pathogenesis of cardiovascular diseases (CVD) in patients with end-stage renal disease (ESRD) [1]. Despite the improvements in diagnostic tools and therapeutic approaches, CVD remains the most common cause of morbidity and mortality in this population [2]. This heightened risk is also found to be ongoing even in ESRD patients who received renal transplantation (Rtx) [3]. As a sign of atherosclerosis, increased intima-media thickness (IMT) of the common carotid artery has been widely used and accepted as a strong predictor of cardiovascular events and mortality in ESRD patients [4]. Several studies demonstrated that systemic persistent inflammation could be the main factor responsible for this increased risk in ESRD patients regardless of the renal replacement therapy [5]. To prove this hypothesis, several biomarkers, including C-reactive protein (CRP), interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α), were studied to assess the relation of inflammation and CVD in general and chronic kidney disease (CKD) populations [5, 6]. White blood cell count and its subtypes were also found to be markers of inflammation in CVD [7]. Recently, the neutrophil-to-lymphocyte ratio (NLR) was introduced as a potential marker to determine inflammation in cardiac and noncardiac disorders [8–10]. NLR was also shown as a predictor of long-term mortality in patients who underwent percutaneous coronary intervention [11]. In addition, we recently demonstrated that NLR could predict inflammation in ESRD patients [12].

Pentraxin-3 (PTX-3) is a novel molecule which has been found to be closely associated in the pathogenesis of atherosclerosis [13]. PTX-3 is produced by various cells including monocytes/macrophages, neutrophils, vascular smooth muscle cells, fibroblasts, and endothelial cells [14]. Recent studies have shown that PTX-3 is also elevated in CKD and associated with endothelial dysfunction and mortality in this population [15, 16]. However, there are conflicting results about the exact role of PTX-3 in atherosclerotic vascular disease. Some investigators have concluded that PTX-3 has atheroprotective effects [17, 18], whereas others have suggested that it could have atherogenetic properties [13, 15].

The role of Rtx in terms of atherogenesis is still unclear. To date, in the literature, there has been no study investigating the relationship between atherosclerosis and PTX-3 in Rtx patients with well-functioning kidneys. Hence, we aimed to investigate the relationship between PTX-3, NLR, and carotid IMT (CIMT) in Rtx patients, and to compare these results with those obtained from healthy subjects.

Materials and Methods

The study protocol was approved by the Medical Ethics Committee of Selcuk University (Meram School of Medicine, Meram, Turkey). Written informed consent was obtained from all subjects included in the study.

This was a cross-sectional study involving 29 Rtx patients (12 females, 17 males; mean age: 40.1 ± 11.9 years) followed at least 6 months in the transplantation unit of Necmettin Erbakan University and 19 healthy subjects (9 females, 10 males; mean age: 36.9 ± 8.9 years) between January and November 2011.

All patients were operated by the same general surgery team in the renal transplantation unit of Necmettin Erbakan University. Patients aged 18–70 years willing to participate in the assessment of CIMT by carotid duplex ultrasonography were screened. A review of the medical records (including information on age, sex, weight, medications, and primary disease of ESRD) was made. Exclusion criteria were: (1) angina pectoris and/or documented coronary artery disease, (2) congestive heart failure, (3) active infection, (4) autoimmune disease, (5) severe secondary hyperparathyroidism (patients with intact parathyroid hormone >500 pg/ml), and (6) nephrotic-range proteinuria. Fifty-five patients were screened and 26 patients excluded from the study. Of the 26 excluded patients, 6 had documented coronary artery

disease, 8 had congestive heart failure (New York Heart Association class III–IV), 7 had active infection, 3 had secondary hyperparathyroidism, and 2 had autoimmune disease. None of the patients included in the study had nephrotic-range proteinuria and arrhythmia based on electrocardiography. The remaining 29 Rtx patients were enrolled in the study. Nineteen age- and sex-matched normotensive healthy individuals referred from outpatient clinics of the Internal Medicine Department of Necmettin Erbakan University were enrolled as control subjects. They were subjected to the same inclusion and exclusion criteria as the patients. The systolic blood pressure and diastolic blood pressure of the patients and healthy subjects were measured in the upright sitting position after ≥5 min of rest using an Erka sphygmomanometer (PMS Instruments Ltd., UK) with an appropriate cuff size. Two readings were recorded for each individual. The mean value of the two readings was defined as the blood pressure. Patients with systolic blood pressure and diastolic blood pressure ≥140 and ≥90 mm Hg, respectively, were assumed to be hypertensive.

Eighteen patients were taking antihypertensive drugs (all patients were on verapamil). Four patients were taking an oral calcium-vitamin D combination, and 5 patients were on active vitamin D. As for maintenance immunosuppressive therapy, of 29 Rtx patients, 29 (100%) were on prednisolone, 20 (68.9%) were on tacrolimus, 23 (79.3%) were on mycophenolate mofetil, 6 (20.7%) were on cyclosporin A, 2 (6.9%) were on sirolimus, and 4 (13.8%) were on everolimus therapy. Six patients (20.7%) were on intensive insulin and 4 (13.8%) were on oral antidiabetic medication. The donor source was living-related in 18 patients (62%), living-unrelated in 2 (7%), and deceased-donor in 9 (31%). None of the patients had transplant nephrectomy.

None of our patients or control subjects were taking dietary supplements and none of the Rtx patients were smoking. Of the healthy subjects, 2 were smoking.

Biochemical Analyses

Venous blood samples were drawn after an overnight fast and stored at –80°C for biochemical and blood count analyses in healthy subjects and Rtx patients. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglyceride, and phosphorus assays were analyzed on the Synchron LX20 system (Beckman Coulter, USA) with the original Beckman reagents. HDL-C levels were determined with the direct enzymatic method without precipitation on Synchron LX20 systems (Beckman Coulter, USA). Low-density lipoprotein cholesterol (LDL-C) levels were calculated with the Friedewald formula [19]. Serum high-sensitivity (hs)-CRP levels were measured with a high-sensitivity immunoturbidimetric assay (Diasis Diagnostic System) using an automated clinical chemistry analyzer. The normal range reference interval of hs-CRP for adults was accepted as <10 mg/l.

Plasma PTX-3 Measurements

In both patients and healthy controls, plasma PTX-3 levels were measured by a commercially available kit based on the quantitative sandwich enzyme immunoassay technique (R&D Systems, Human Pentraxin 3/TSG-14 Immunoassay kit, Cat No. DPTX3). The calculated overall intra-assay coefficient of variation of PTX-3 was 4.4%.

Definition of NLR

Complete blood counts with automated differential counts, which included total white blood cells, neutrophils, and lymphocytes, were obtained at the time of admission. NLR was calculated as the ratio of neutrophils and lymphocytes, both obtained from the same automated blood sample at the admission of the study.

Glomerular Filtration Rate Assessment

Glomerular filtration rate (GFR) was calculated according to the simplified version of the Modification of Diet in Renal Disease (MDRD) study prediction equation formula, $GFR = 186 \times \text{creatinine}^{-1.154} \times \text{age}^{-0.203} \times 1.212$ (if African-American) $\times 0.742$ (if female), as defined by Levey et al. [20].

CIMT Measurements

The CIMT recordings were performed by a single investigator (H.O.), who was blinded to the two groups. The carotid arteries were evaluated with the Vivid 7 echocardiography device (General Electrics, Horten, Norway) using a 10-MHz linear probe. The acquired images were recorded for playback analysis and were later measured off-line. The common carotid artery, the carotid bulb, and internal and external

carotid arteries were visualized on both sides. The IMT of the carotid arteries was measured in the distal common carotid artery at a level 15–20 mm proximal to the carotid bulb. The two bright echogenic lines in the arterial wall were identified as the intima and the media. Three measurements were made for each side of the body; separate means were calculated and recorded as the right and left IMT. The intraobserver coefficient of variation for CIMT was 2.0%.

Statistical Analyses

Statistical analyses were carried out using the Statistical Package for Social Sciences for Windows version 15.0 (SPSS, Chicago, Ill., USA). Data were expressed as means ± SD, with a significance level of $p < 0.05$. The normal distributions of all variables were tested using the Kolmogorov-Smirnov test. Dichotomous variables were compared using a χ^2 test. Statistical differences between parametric data of the two groups were analyzed using Student's t test. The Mann-Whitney U test was used to determine differences between nonparametric data. The nonparametric Spearman coefficient of correlation was used to assess correlations between variables without normal distribution. Significant determinants identified from univariate analysis were studied in a stepwise multiple regression model.

Results

Patients' Baseline Characteristics

The baseline characteristics of the 29 Rtx patients and 19 healthy subjects are depicted in table 1 with univariate correlates with CIMT including all participants. The etiologies of the Rtx patients were chronic glomerulonephritis (n = 6, 20%), diabetic nephropathy (n = 3, 10%), hypertensive nephropathy (n = 4, 13.3%), polycystic kidney disease (n = 1, 3.3%), and unknown (n = 15, 50%). Of the 29 Rtx patients, 14 (48%) had posttransplant hypertension, 9 (31%) had posttransplant dyslipidemia, and 11 (38%) had new-onset diabetes after transplantation. There were no differences in age; gender; BMI; systolic and diastolic blood pressure; biochemical parameters including serum glucose, albumin, LDL-C, and HDL-C; aspartate transaminase; alanine transaminase, and phosphorus between Rtx patients and healthy subjects. Spot urine protein-to-creatinine ratio values of healthy controls and Rtx patients were 0.09 ± 0.1 and 0.3 ± 0.4, respectively ($p = 0.004$). Serum uric acid, triglyceride, and calcium were significantly higher in Rtx patients compared to controls ($p < 0.0001$, $p < 0.001$, and $p < 0.001$, respectively). Estimated GFR (eGFR) measurements were also found to be significantly lower in Rtx patients than healthy subjects (62.6 ± 24.2 vs. 121.2 ± 28.3, respectively, $p < 0.0001$).

PTX-3, NLR, hs-CRP, and CIMT Measurements

Results of inflammation and atherosclerosis markers including PTX-3, hs-CRP, NLR, and CIMT are shown in table 1. Serum PTX-3 and hs-CRP levels of the Rtx patients were found to be significantly higher compared to healthy subjects ($p < 0.0001$, for both). Neutrophil count and NLR were also significantly higher in Rtx patients as compared with healthy controls ($p < 0.0001$, for both). The mean CIMT of Rtx patients and healthy subjects were 0.75 ± 0.20 and 0.4 ± 0.08 mm, respectively ($p < 0.0001$; table 1).

The Rtx patients were divided into two subgroups according to CIMT value (group 1, CIMT <0.82 mm; group 2, CIMT ≥0.82 mm). Age, eGFR, spot urine protein-to-creatinine ratio, serum LDL-C and HDL-C, triglyceride, and PTX-3 levels were similar in both group 1 and 2 Rtx patients (table 2). However, serum glucose and hs-CRP levels were found to be significantly higher in group 2 patients compared to group 1 patients ($p = 0.001$ and $p < 0.0001$, respectively).

Table 1. Demographic, clinical, and laboratory features of healthy subjects and Rtx patients

Parameters	Healthy subjects (n = 19)	Rtx patients (n = 29)	p	ρ (p)[1]
Age, years	36.9 ± 8.9	40.1 ± 11.9	0.33	0.29 (0.05)
Male/female, n	10/9	17/12	0.80	–
BMI	26.7 ± 4.3	25.0 ± 3.9	0.18	–
SBP, mm Hg	133 ± 9	124 ± 31	0.56	–
DBP, mm Hg	83 ± 8	85 ± 14	0.94	–
Hemoglobin, g/dl	13.8 ± 0.9	13.6 ± 1.8	0.87	–
Glucose, mg/dl	92.3 ± 8.2	110 ± 38.9	0.10	–
Urea, mg/dl	27.2 ± 8.22	41.8 ± 28	0.005	–
Creatinine, mg/dl	0.7 ± 0.15	1.4 ± 0.6	<0.0001	0.66 (<0.0001)
Albumin, g/dl	4.4 ± 0.2	4.3 ± 0.3	0.27	–0.19 (0.20)
Uric acid, mg/dl	3.9 ± 0.7	5.7 ± 1.5	<0.0001	0.60 (<0.0001)
AST, IU/l	22 ± 9	22 ± 6	0.60	–
ALT, IU/l	21 ± 17	24 ± 9	0.70	–
LDL-C, mg/dl	124 ± 27	108 ± 34	0.16	0.06 (0.69)
HDL-C, mg/dl	47 ± 13	46 ± 13	0.85	0.05 (0.7)
Triglyceride, mg/dl	104 ± 36.7	185 ± 84.8	0.001	0.37 (0.01)
Calcium, mg/dl	8.8 ± 0.58	9.6 ± 0.67	0.001	–
Phosphorus, mg/dl	3.0 ± 0.4	3.0 ± 0.86	0.65	–
eGFR, ml/min	121.2 ± 28.3	62.6 ± 24.2	<0.0001	–0.69 (<0.0001)
hs-CRP, mg/l	2.8 (2.0–3.6)	8.4 (4.15–12.8)	<0.0001	0.64 (<0.0001)
Spot urine protein-to-creatinine ratio, g/mg	0.09 ± 0.1	0.3 ± 0.4	0.004	0.50 (0.001)
PTX, ng/ml	2.3 (2.1–2.5)	5.2 (4.45–6.30)	<0.0001	0.68 (<0.0001)
Neutrophils, mm^3	3,213 ± 720	5,429 ± 2,173	<0.0001	
Lymphocytes, mm^3	2,409 ± 742	2,201 ± 691	0.48	
NLR	1.28 ± 0.33	2.6 ± 1.31	<0.0001	0.64 (<0.0001)
CIMT, mm	0.4 ± 0.08	0.75 ± 0.20	<0.0001	–

Values are means ± SD or medians (interquartile range), unless otherwise indicated. ALT = Alanine aminotransferase; AST = aspartate aminotransferase; DBP = diastolic blood pressure; SBP = systolic blood pressure.

[1] Spearman rank univariate analysis for CIMT.

Univariate Correlations between CIMT and Continuous Variables

In univariate analysis, CIMT was positively correlated with age, BMI, serum phosphorus, and LDL in Rtx patients (table 3). When all participants were studied, CIMT was positively correlated with age, serum creatinine, uric acid, and triglyceride, and negatively correlated with GFR (table 1).

Serum uric acid levels were positively correlated with hs-CRP ($r = 0.37$, $p = 0.047$), but not with PTX-3 ($r = 0.14$, $p = 0.48$), in Rtx patients.

Univariate Correlations between CIMT and Inflammatory Parameters

To investigate the relationship between atherosclerosis and inflammation, we performed a univariate correlation analysis. CIMT was positively correlated with PTX-3 and NLR when all participants were included ($r = 0.68$, $p < 0.0001$ and $r = 0.64$, $p < 0.0001$, respectively). However, in Rtx patients, NLR and PTX-3 were not found to be correlated with CIMT (data not shown). In contrast, hs-CRP was found to be positively correlated with CIMT in Rtx patients.

Table 2. Demographic features and biochemical values of Rtx patients according to the CIMT group

Parameters	Group 1 CIMT <0.82 (n = 19)	Group 2 CIMT ≥0.82 (n = 10)	p value
Age, years	37.3 ± 12.0	45.3 ± 10.2	0.09
Male, n (%)	10 (52.6)	7 (70)	0.31
HT, n (%)	11 (58)	5 (50)	0.40
DM, n (%)	0 (0)	3 (33)	0.04
BMI	24.3 ± 3.9	26.5 ± 3.6	0.10
eGFR, ml/min	67.2 ± 25.1	53.9 ± 20.7	0.27
Glucose, mg/dl	95 ± 21	138 ± 49	0.001
Creatinine, mg/dl	1.29 ± 0.65	1.43 ± 0.43	0.28
Uric acid, mg/dl	5.5 ± 1.37	6.1 ± 1.62	0.32
Calcium, mg/dl	9.7 ± 0.66	9.5 ± 0.70	0.60
Phosphorus, mg/dl	2.8 ± 0.6	3.4 ± 1.1	0.03
Albumin, g/dl	4.3 ± 0.28	4.4 ± 0.29	0.50
Triglyceride, mg/dl	189 ± 92	178 ± 74	0.55
HDL, mg/dl	45 ± 12	48 ± 16	0.74
LDL, mg/dl	103 ± 26	116 ± 46	0.14
NLR	2.34 ± 0.98	3.2 ± 1.72	0.19
hs-CRP, mg/l	6.8 ± 4.8	15.5 ± 9.3	0.004
PTX-3, ng/ml	5.8 ± 2.1	5.5 ± 1.40	0.91
Procalcitonin, ng/ml	0.11 ± 0.13	0.09 ± 0.05	0.30
Spot urine protein-to-creatinine ratio, g/mg	0.20 ± 0.20	0.52 ± 0.60	0.08

Values are means ± SD or n (%). DM = Diabetes mellitus; HT = hypertension.

Table 3. Correlation of CIMT and other continuous variables in Rtx patients

Parameters	r	p value
Age (years)	0.54	0.003
BMI	0.38	0.04
Phosphorus (mg/dl)	0.50	0.006
LDL (mg/dl)	0.38	0.04
hs-CRP (mg/l)	0.44	0.02

r = Correlation coefficient.

Predictors of CIMT in Rtx Patients

The multivariate linear regression analysis revealed that only hs-CRP was found to be an independent variable of increased CIMT. Regression results are shown in table 4.

Discussion

This cross-sectional study mainly showed that inflammation measured by serum PTX-3, hs-CRP levels, and NLR and atherosclerosis defined by CIMT persisted in Rtx patients despite a significant improvement in kidney function. However, unlike hs-CRP, PTX-3 and NLR failed to predict CIMT in this population. Besides hs-CRP, traditional Framingham

Table 4. Stepwise regression analysis to determine the independent predictors of CIMT in Rtx patients

Parameters	Standardized beta[1]	p value	95% CI
Step 1[2]			
Age (years)	0.17	0.38	–0.09 to 0.02
BMI	0.10	0.62	–0.04 to 0.07
Phosphorus (mg/dl)	0.20	0.30	–0.10 to 0.34
LDL (mg/dl)	0.09	0.60	–0.004 to 0.006
hs-CRP (mg/l)	0.45	0.02	0.006 to 0.05
Step 4[3]			
hs-CRP (mg/l)	0.54	0.003	0.013 to 0.054

[1] Standardized beta: standardized regression coefficients of the variables.
[2] Adjusted $r^2 = 0.29$, $p = 0.023$; was defined to model statistics for each step.
[3] Adjusted $r^2 = 0.26$, $p = 0.003$; was defined to model statistics for each step.

risk factors including advanced age, obesity, LDL levels, and high phosphorus were found to positively associate with CIMT in Rtx patients without known CVD.

Ultrasonographically measured CIMT was found to be a well-validated marker of atherosclerosis as well as a commonly used surrogate endpoint in clinical trials [21]. The CIMT of our patients was also significantly high compared to healthy controls. Thus, by virtue of ongoing inflammation, our Rtx patients might have more atherosclerotic vessels than the controls. We also showed that CIMT was positively correlated with age, BMI, serum phosphorus, LDL-C, and hs-CRP in Rtx patients. When both healthy subjects and Rtx patients were included in the model, CIMT was found to be positively associated with age, serum creatinine, uric acid, triglyceride, hs-CRP, and spot urine protein-to-creatinine ratio, and negatively correlated with eGFR. Our results are partially in accord with the study investigating the predictors of CIMT in a large CKD cohort [22]. In this study, serum hs-CRP, uric acid, phosphorus, intact parathyroid hormone, $Ca \times PO_4$ product, HOMA-insulin resistance, and mean arterial pressure were found to be positively correlated, whereas eGFR and serum calcium levels were negatively correlated with CIMT in CKD stage 1–5 patients [22]. The authors also showed that CIMT values were significantly decreased 30 and 90 days after Rtx in subgroup analysis [22]. Multivariate analysis of our study revealed that only hs-CRP was found to be an independent predictor of increased CIMT in Rtx patients.

Recently, Hornum et al. [23] investigated the effect of Rtx on arterial function, and they showed a significant improvement regarding endothelial function, mean arterial pressure, and plasma von Willebrand factor levels, but not in plasma CRP and albumin levels. Seyahi et al. [24] also demonstrated that Rtx did not reverse or stop coronary artery calcification in this population. Therefore, one might hypothesize that Rtx may not completely reverse nontraditional risk factors including inflammation, oxidative stress, and atherosclerosis.

PTX-3, a member of long pentraxins, acts as an immunomodulator involved in the humoral arm of immunity [25] and also participates in inflammation, angiogenesis, and CVD [26]. PTX-3 can be synthesized by various cells including myeloid dendritic cells, neutrophils, macrophages, fibroblasts, endothelial cells, and vascular smooth muscle cells [14]. Growing evidence suggests that PTX-3 is one of the most rapid inflammatory markers circulating in the early phase of inflammation milieu [27]. In 2007, Tong et al. [28] showed that PTX-3 levels were increased in CKD patients. According to this study, CKD patients with high PTX-3 levels had higher cardiovascular mortality. The same group also demonstrated that PTX-3 and proteinuria were independently associated with endothelial dysfunction in type 2 diabetic patients

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[15]. Moreover, short-term blockage of the renin-angiotensin-aldosterone system with angiotensin-converting enzyme inhibitor treatment improves endothelial function, diminishes proteinuria, and normalizes PTX-3 and hs-CRP [29]. In the present study, both PTX-3 and hs-CRP levels were higher in Rtx patients compared to healthy subjects. Creatinine clearances measured by eGFR of our Rtx patients were similar to those of patients with CKD stage 2. Despite the improvement of kidney function in this population, GFR places them in one of the stages of CKD. Therefore, Rtx patients might be considered a subset of CKD patients. This may partly explain why Rtx patients had high PTX-3 and hs-CRP levels. In the present study, PTX-3 levels were found to be positively correlated with CIMT in all subjects. However, we failed to demonstrate PTX-3 as an independent predictor of CIMT in Rtx patients.

There has been a debate regarding the exact role of PTX-3 in inflammation and atherosclerosis. Some researchers have suggested that PTX-3 might have a cardioprotective role as shown in a mouse model of acute myocardial infarction [17]. According to results of this study, in wild-type mice, PTX-3 levels were increased after the onset of acute myocardial injury; however, ptx-3-deficient mice showed a larger infarction area on the myocardium, which was associated with increased neutrophil infiltration, apoptosis of cardiomyocytes, and serum IL-6 levels, and no reflow area in the heart. In addition, to prove the cardioprotective effect of PTX-3, ptx-3-deficient and wild-type mice were treated with human recombinant PTX-3. They also demonstrated that human recombinant PTX-3 therapy significantly reduces the extent of cardiac damage and IL-6 levels in ptx-3-deficient mice. The expression of PTX-3 was also found to be induced by HDL in human and mice endothelial cells [30]. Furthermore, Deban et al. [31] reported that PTX-3 could have a role in dampening excessive neutrophil recruitment, which may lessen inflammation. All of these findings were strengthened by the observation that PTX-3 deficiency is also associated with increased atherosclerosis and macrophage infiltration in these atherosclerotic lesions in apolipoprotein E-deficient mice [18].

Currently, the exact role of PTX-3 is unknown in both the CKD and Rtx population. On the basis of the experimental and clinical data mentioned above, we hypothesized that PTX-3 might have an endothelial protective effect rather than a deleterious effect in Rtx patients. The main basis of our hypothesis was that endothelial function was found to be much more improved in Rtx patients compared to CKD patients despite ongoing inflammation in this population. Hence, one might hypothesize that kidney transplantation may not completely reverse nontraditional risk factors including inflammation. This may partly explain why Rtx patients had high PTX-3 and hs-CRP levels compared to healthy subjects.

Elevated serum uric acid levels have also been associated with increased inflammation in vascular cells [32, 33]. In a meta-analysis, hyperuricemia was found be independently associated with increased CVD [34]. Recently, Kanbay et al. [35] demonstrated that serum uric acid and PTX-3 levels were independently associated with CAD in the early stages of CKD. They also showed that hyperuricemic subjects have worse endothelial function and that treatment of hyperuricemia with allopurinol improves endothelial dysfunction. In the present study, serum uric acid levels were significantly higher in Rtx patients compared to controls. Serum uric acid levels were positively correlated with CIMT in our study when all participants were included. However, we could not find any correlation between uric acid and CIMT in Rtx patients. This result is in accord with a previous study done by Yilmaz et al. [22]. They also showed that serum uric acid levels of Rtx patients were higher compared to healthy subjects; however, in univariate analysis, they failed to show that uric acid was correlated with CIMT in Rtx patients. In the present study, serum uric acid levels were also positively correlated with hs-CRP, but not with PTX-3, in Rtx patients. None of our patients have symptomatic gout; however, patients with high uric acid levels also have increased atherosclerosis and inflammation.

The calculation of NLR is a very simple and cheap method when compared with the other inflammatory cytokines including IL-6, IL-1β, and TNF-α. White blood cell count and its subtypes are known as classic markers of inflammation in CVD [7]. In recent years, neutrophilia and relative lymphocytopenia were shown to be independent predictors of mortality in patients with acute heart failure [36, 37]. Moreover, NLR was introduced as a potential marker to determine inflammation in cardiac and noncardiac disorders [8–10]. Additionally, NLR was shown as a predictor of long-term mortality in patients who underwent percutaneous coronary intervention [11]. In our previous study, we demonstrated that NLR was positively correlated with TNF-α in ESRD patients [12]. To date, in the literature, no study has evaluated NLR and its association with atherosclerosis in Rtx patients. In the present study, Rtx patients had higher neutrophil counts and NLR compared to healthy subjects. Mean neutrophil counts were slightly elevated in Rtx patients. We believe that this increase in neutrophil counts was regardless of using corticosteroid because all of our patients were using low-dose prednisolone (5 mg/day) that did not alter the circulating amount of neutrophils. Although Rtx patients were also using lymphocyte-depleting agents as a maintenance immunosuppressive therapy, there was no significant difference between Rtx patients and controls in terms of lymphocyte counts. Thus, increased NLR might be related to ongoing inflammation in this population.

Our study had some limitations. First, the study sample was relatively small. Second, since this is a cross-sectional study, we cannot draw a cause-and-effect relationship from our findings.

In conclusion, this study shows that inflammation and atherosclerosis persist in Rtx patients without known CVD. Rtx is the preferred replacement therapy in ESRD patients all over the world; however, this option cannot completely cure all metabolic disturbances in this population. Further randomized controlled trials investigating the exact role of PTX-3 in the pathogenesis of atherosclerosis in Rtx patients are needed.

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Disclosure Statement

None of the authors have reported conflicts of interest.

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