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Uricemia and homocysteinemia: nontraditional risk factors in the early stages of chronic kidney disease – Preliminary data

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Abstract. – BACKGROUND: Patients with chronic kidney disease (CKD) show a risk of cardiovascular death, which is 10-100 times higher than that in the general population. This increase is not completely explained by the traditional cardiovascular risk factors. Hyperuricemia and hyperhomocysteinemia are highly prevalent in CKD. Patients suffering from these complications present accelerated atherosclerosis, determined mainly from the endothelial dysfunction that carries out a central role in the pathogenesis of cardiovascular diseases.

AIM: The hypothesis was that brachial artery flow mediated dilation (FMD) and carotid intima-media thickness (cIMT) evaluation can be considered as early and systemic markers of atherosclerosis and that nontraditional risk factors, such as hyperhomocysteinemia and hyperuricemia, are associated with early endothelial dysfunction and vascular damage in patients suffering from first- and second-stage CKD.

PATIENTS AND METHODS: The study comprised 50 patients, 10 for each CKD stage, and 15 age- and sex-matched healthy controls. We compared the traditional and nontraditional factors for cardiovascular diseases with alterations of vascular reactivity, such as cIMT, and brachial artery FMD, in patients affected by CKD with those in the control group.

RESULTS: In our study, hyperuricemia was significantly and independently associated with brachial artery FMD reduction ($p = 0.007$), while hyperhomocysteinemia was significantly and independently associated with carotid intima-media thickening ($p = 0.021$) in patients at Stage I and II KDOQI (Kidney Disease Outcomes Quality Initiative).

CONCLUSIONS: In our study, we found a progressive increase in the inflammatory indices and endothelial dysfunction at the early stages of CKD. Hyperuricemia and hyperhomocysteinemia were associated with IMT and FMD at Stage I-III KDOQI, and can be used as markers of subclinical atherosclerosis, especially in nephropathic patients with high cardiovascular risk.

Key Words:
Carotid intima-media thickness, Brachial artery flow-mediated dilation, Endothelial dysfunction, Chronic kidney disease, Uricemia and homocysteinemia.

Introduction

Cardiovascular disease is the most frequent cause of morbidity and mortality in patients with chronic kidney disease (CKD), and their risk of cardiovascular death is 10-100 times higher than the general population[1]. This increase is not completely explained by traditional cardiovascular risk factors. Chronic renal failure (CRF) is frequently associated with increased plasma levels of homocysteine (Hcy), an amino acid that can be considered as a new uremic toxin. Thus, hyperhomocysteinemia was shown to be associated with cardiovascular events both in the general population and CRF patients[2-4]. Hyperuricemia is highly prevalent in CKD and may have a role as a uremia-related cardiovascular risk factor. Putative mechanisms include inflammation, endothelial dysfunction, and vascular smooth muscle proliferation[5-6].

The endothelial dysfunction has a central role in the pathogenesis of cardiovascular diseases, and there is a strict association between endothelial dysfunction, inflammation, and atherosclerosis[7]. The endothelium can be considered as a real organ with autocrine and paracrine action. The main chemical endothelial mediators are nitric oxide (NO), prostacyclin, endothelium derived hyperpolarizing factor (EDHF), endothelin-1, thromboxane A2, A2 prostaglandin, platelet activating factor (PAF), etc. Among these mediators, NO is the most important in the control of the arterial and

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microcircle elasticity, both in baseline conditions and after stimulation. Endothelium agonist substances, such as acetylcholine, bradykinin, substance P, serotonin, and shear stress determine the release of NO with short half-life (6-7 s), which is capable of inducing smooth musculature relaxation and vasodilatation[7]. The alteration of the endocrine-paracrine activity of endothelium is responsible for the endothelial dysfunction[8]. Cardiovascular risk factors promote the development of endothelial dysfunction, characterized by impairment of endothelium-dependent vasodilatation and pro-coagulant/pro-inflammatory endothelial activities. The endothelial function can be assessed with brachial artery endothelium-dependent flow-mediated dilatation (FMD). NO is the principal mediator of FMD generated from the activation of endothelial nitric oxide synthetase (eNOS); thus, the FMD can be detected only in the presence of integral endothelium[9]. Another early sign of an accelerated atherosclerotic process is evaluated by carotid intima-media thickness (cIMT), which is capable of predicting the cardiovascular events in the general population, correlated to Framingham score[10-12]. Furthermore, hyperuricemia and hyperhomocysteinemia are highly prevalent in CKD and may have a role as uremic toxins and nontraditional cardiovascular risk factors in these patients.

The aim of the present study was to determine the importance of cIMT and brachial artery FMD as an initial marker of atherosclerosis in patients at different stages of kidney disease. We compared the traditional and nontraditional factors for cardiovascular diseases with alterations in the vascular reactivity in patients affected by CKD (Stage I-V KDOQI: Quality Disease Outcomes Quality Initiative) with those in a homogenous age- and sex-matched control group. The hypothesis was that FMD and cIMT evaluation can be considered as early and systemic markers of atherosclerosis, and that nontraditional risk factors, such as hyperhomocysteinemia and hyperuricemia, are associated with early endothelial dysfunction and vascular damage in patients at first and second stage of chronic renal insufficiency.

Patients and Methods

A total of 50 CKD patients were enrolled in this study (26 females and 24 males), with a median age of 52.9 years. Furthermore, 10 patients from each stage of CKD (Stage I-V), according to the KDOQI guidelines, and 15 age- and sex-matched healthy controls were also included. The eGFR (estimated Glomerular Filtration Rate) was calculated using the abbreviated Modification of Diet in Renal Disease formula, as defined by Levey et al[13].

We excluded patients who suffered from infection, active inflammation, autoimmune disease, malignancy, heart failure, atrial fibrillation, acute myocardial infarction, valvular heart disease, cerebrovascular disease, common carotid artery (CCA) stenosis, and acute coronary syndrome within 3 months prior to the study. In addition, patients with a previous diagnosis of gout, diabetes mellitus, current use of oral antidiabetic medication, insulin, thiazide, allopurinol or uricosuric, nephrotic syndrome, or with a history of smoking were excluded.

The etiology of CKD in these patients was chronic glomerulonephritis in 12 cases, nephrosclerosis in 16 cases, chronic pyelonephritis in 3 cases, reflux nephropathy in 3 cases, autosomal polycystic kidney disease in 4 cases, and unknown in 12 cases.

Antihypertensive, antiplatelet, and statin therapies were continued in the patients included in the study.

Blood Pressure Measurements

Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg or diastolic BP (DBP) ≥ 90 mmHg on repeated measurements or the use of antihypertensive drugs. Arterial BPs were measured by a physician three times after a 15-min resting period in the morning, and the mean values were calculated for SBP and DBP for all participants.

Laboratory Measurements

Blood sampling was performed in the morning, after 12 h of fasting. Samples (6-8 ml) of venous blood were collected from each subject in the morning between 08:00 and 09:00, after an overnight fast. After clot formation, the samples were centrifuged (4000 rpm) at room temperature for 10 min. The serum samples were stored at –80°C until the time of the assay.

In all patients, we measured the levels of fasting plasma glucose, total serum cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), creatinine, blood urea nitrogen (BUN), calcemia, phosphate, albumin, serum electrolytes, VES, PCR, and fibrinogen. Furthermore, 24-h urine collection was per-

formed three times and the average of the three 24-h proteinuria measurements was taken as the representative of each participant's 24-h protein excretion rate.

Vascular Assessment

According to Celermajer et al[14], the endothelium-dependent vasodilation (FMD) of the brachial artery was assessed by using high-resolution ultrasound. The subjects were investigated in the Vascular Laboratory suite under standardized conditions, by a single investigator. The patients were instructed to fast for at least 8-12 h before the study, and they were examined in a quiet, temperature-controlled room. All vasoactive medications were withheld for at least four half-lives, if possible. In addition, the subjects were asked to stop exercising and avoid intake of substances that might affect FMD, such as caffeine, high-fat foods, vitamin C, and tobacco for at least 4-6 h before the study. Ultrasound Toshiba XP5 (Japan) with linear probe from 7.5 to 10 MHz was used for the ultrasonographic study of FMD. The timing of each image frame with respect to the cardiac cycle was determined with simultaneous electrocardiography (ECG) recording on the ultrasound system video monitor. The subject was positioned supine with arms in a comfortable position for imaging the brachial artery. The brachial artery was imaged above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall was selected for continuous 2D gray-scale imaging. To create a flow stimulus in the brachial artery, a sphygmomanometric cuff was first placed on the forearm. Typically, the cuff was inflated to at least 50 mmHg above the SBP to occlude arterial inflow for a standardized length of time. The release of the occluding cuff resulted in reactive hand hyperemia and an associated increase in blood flow through the brachial artery, which induced shear stress on the arterial wall and provided a stimulus for endothelium-dependent dilatation. Brachial artery diameter following reactive hyperemia was recorded for 5 min after tourniquet release. Flow-mediated vasodilatation was typically expressed as the change in post-stimulus diameter as a percentage of the baseline diameter[15].

$$\text{FMD} = \frac{(\text{Diameter post-hyperemia} - \text{basal diameter})}{\text{Basal diameter}} \times 100$$

The values of FMD were considered normal if they were greater than 10%.

Assessment of FMD of the brachial artery has increased in clinical trials because of its easy-to-use, efficient, and noninvasive nature, and presents the biological and technical variability of the measurement[16].

Common carotid-artery imaging is performed by a single investigator in the Vascular Laboratory suite under standardized conditions with high-resolution B-mode ultrasonography Toshiba XP5 with linear probe from 7.5 to 10 MHz. One longitudinal image of the common carotid artery and three longitudinal images of the internal carotid artery are acquired. The maximal IMT of the common carotid artery and the internal carotid artery is defined as the mean of the maximal IMT of the near and far wall on both the left and right sides. Thus, the measurements available for averaging range from 1 to 4 for the common carotid artery and from 1 to 12 for the internal carotid artery. The mean cIMT is defined as the mean of ICA, BIF, and the three highest CCA measurements[17-18].

Statistical Analysis

Data management and analysis were performed using IBM® SPSS® Statistics 18 for Windows® software. The normality of the variables was tested using the Kolmogorov-Smirnov test for normal distributions. All continuous variables following a normal distribution were expressed as mean ± standard deviation, and those that are not normal were expressed as median (IQR). Categorical variables were expressed as number (percentage). Pearson's or Spearman's correlation was used to determine the relationship and strength of the association between FMD or IMT and other variables. Univariate and multivariate linear regression was performed between FMD or IMT and parametric variables that reached statistical significance ($p < 0.05$) in the bivariate correlation. Multivariate linear regression analysis was performed to determine independent correlations between FMD or IMT and other variables by using a stepwise approach.

We performed multivariate linear regression analysis to determine the independent predictors of endothelium-dependent FMD and IMT adjusted for traditional risk factors, such as age, body mass index (BMI), gender, smoking status, and hypertension (≥ 140/90 mmHg or on antihypertensives) in all patients.

Results

As shown in Table I, in the patients at Stage I-III, the uricemia levels were negatively correlated to FMD ($r = -0.48$; $p = 0.007$), but positively correlated to IMT ($r = 0.47$; $p = 0.008$). Furthermore, Hcy levels were positively correlated to cIMT ($r = 0.42$; $p = 0.021$).

The mean age of patients was 53.4 (± 12.8) years. There were no significant differences in age, gender, and BMI between the groups. The mean values of IMT, Hcy, uric acid, and Ca *P (calcium x phosphate product) increased with the progression of renal failure, showing statistically significant differences between the groups (IMT: $F = 53.8$, $p < 0.001$; Hcy: $F = 18.5$, $p < 0.001$; uremia: $F = 18.4$, $p < 0.001$; Ca *P: $F = 12.9$, $p < 0.001$). The mean values of FMD were higher in patients with preserved renal function, showing statistically significant differences between the groups ($F = 61.9$, $p < 0.001$). IMT correlated positively to plasma Hcy ($r = 0.841$, $p < 0.001$). A linear correlation was found between IMT and uric acid ($\beta = 0.170$, $p = 0.019$), and between Ca *P ($\beta = 0.346$, $p < 0.001$) and hemoglobin ($\beta = -0.438$, $p < 0.001$). On the other hand, FMD correlated negatively to Hcy ($r = -0.814$, $p < 0.001$). Multivariate analysis showed a linear correlation between FMD and serum uric acid ($\beta = -0.395$, $p < 0.001$) and Ca *P ($\beta = -0.954$, $p = 0.012$). There was also a significant correlation with some indices of inflammation, such as C-reactive protein (CRP), fibrinogen, and ferritin (Figures 1 and 2, Table I, $p < 0.001$). None of the patients had metabolic syndrome. Were not assayed insulin levels.

Discussion

Cardiovascular mortality in patients with CKD appears to 50% higher than that in the general population, and this seems to be correlated to the chronic systemic flogosis observed in this pathology, which is responsible for endothelial damage, representing the onset of the development of atherosclerotic disease[12]. The association between inflammation and atherosclerosis has been confirmed from observational studies on a large number of patients, which evidenced a strict correlation between elevated serum levels of acute phase proteins, in particular that of the PCR, and increased cardiovascular risk[19,20]. However, the role of hyperuricemia in the process of atherosclerosis is controversial[5]. About two-thirds of uric acid that is generated daily is excreted through kidneys, and

Table I. Relationships between FMD or IMT and other variables.

	FMD		IMT	
	Correlation coefficient	*p*-value	Correlation coefficient	*p*-value
Male	0.082	0.515	-0.031	0.809
Age	0.076	0.550	0.290*	0.019
Hb	0.539**	< 0.001	-0.779**	< 0.001
Serum creatinine	-0.861**	< 0.001	0.764**	< 0.001
BUN	-0.726**	< 0.001	0.824**	< 0.001
Calcium	-0.267*	0.031	0.267	0.058
Phosphorus	-0.562**	< 0.001	0.682**	< 0.001
CaXP	-0.645**	< 0.001	0.757**	< 0.001
Triglyceride	-0.308**	0.013	0.314*	0.011
Glycemia	-0.145	0.251	0.069	0.562
Total cholesterol	-0.049	0.697	0.140	0.267
Serum iron	0.151	0.230	-0.198	0.115
Serum ferritin	-0.609**	< 0.001	0.629**	< 0.001
Serum transferrin	0.464**	< 0.001	-0.469**	< 0.001
Serum uric acid	-0.671**	< 0.001	0.632**	< 0.001
CRP	-0.707**	< 0.001	0.756**	< 0.001
Homocysteine	-0.814**	< 0.001	0.841**	< 0.001
Albumin	0.388*	0.001	-0.383**	0.002
Fibrinogen	-0.574**	< 0.001	0.584**	< 0.001

*Correlation is significant at the level 0.05 (2-tailed). **Correlation is significant at the level 0.01 (2-tailed). Abbreviations: FMD: flow-mediated dilation; IMT: intima media thickness; Hb: hemoglobin; BUN: blood urea nitrogen; CaXP: calcium X phosphate product; CRP: C-reactive protein.

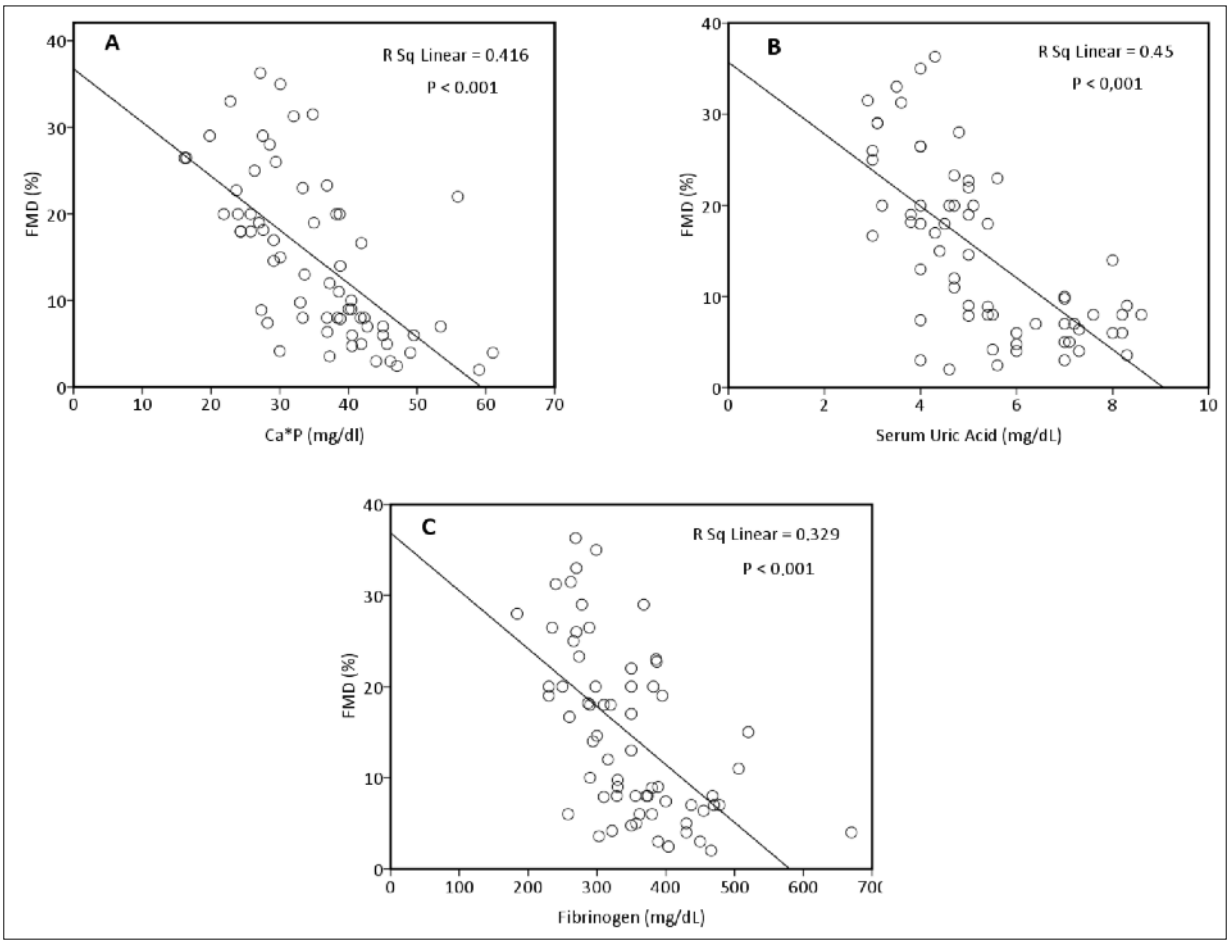

Figure 1. Linear regression plots of: ***(A)*** FMD vs serum Ca*P, r = –0.645, $p < 0.001$; ***(B)*** FMT vs Serum uric acid, r = –0.671, $p < 0.001$; ***(C)*** FMD vs Fibrinogen, r = –0.574, $p < 0.001$. *Abbreviations:* FMD, flow-mediated dilation; Ca*P, calcium X phosphate product.

patients with CKD develop hyperuricemia as the glomerular filtration rate (GFR) declines (Figure 1). Hyperuricemia might play a causative role in oxidative stress, inflammation, and atherosclerosis[21]. In different randomized controlled trials, allopurinol treatment resulted in improvement of oxidative stress, endothelial function, and progression of kidney disease. In another report on Stage III-IV CKD[5] patients, hyperuricemia appeared to be an independent risk factor for all cause and cardiovascular disease mortality.

Hyperuricemia is highly prevalent in CKD, and is gaining interest as a potentially modifiable cardiovascular disease risk factor in the high-risk patient population. Furthermore these patients are at high risk of developing a insulin resistance and metabolic syndrome.

In our study, hyperuricemia and hyperhomocysteinemia were negatively associated with brachial artery FMD and positively associated with cIMT and inflammatory markers. As these alterations already exist in Stage I and II CKD, they can be considered as early markers of endothelial dysfunction and atherosclerosis.

Mild hyperhomocysteinemia is an independent risk factor for increased carotid artery wall thickness.

Several mechanisms for Hcy-associated vascular disease have been proposed, including potentiation of atherosclerosis through endothelial dysfunction, smooth muscle cell hyperplasia, and increased production of oxidized lipids. The complex interaction between hyperhomocysteinemia, oxidative stress, and microinflammation may result in accelerated and early atherosclerosis seen in CRF[22]. Previous carotid B-mode ultrasound studies[2] suggested that hyperhomocysteinemia may play a role in the progression of atherosclerosis, and that elevated plasma Hcy level in CRF is associated with cIMT (Figures 1 and 2).

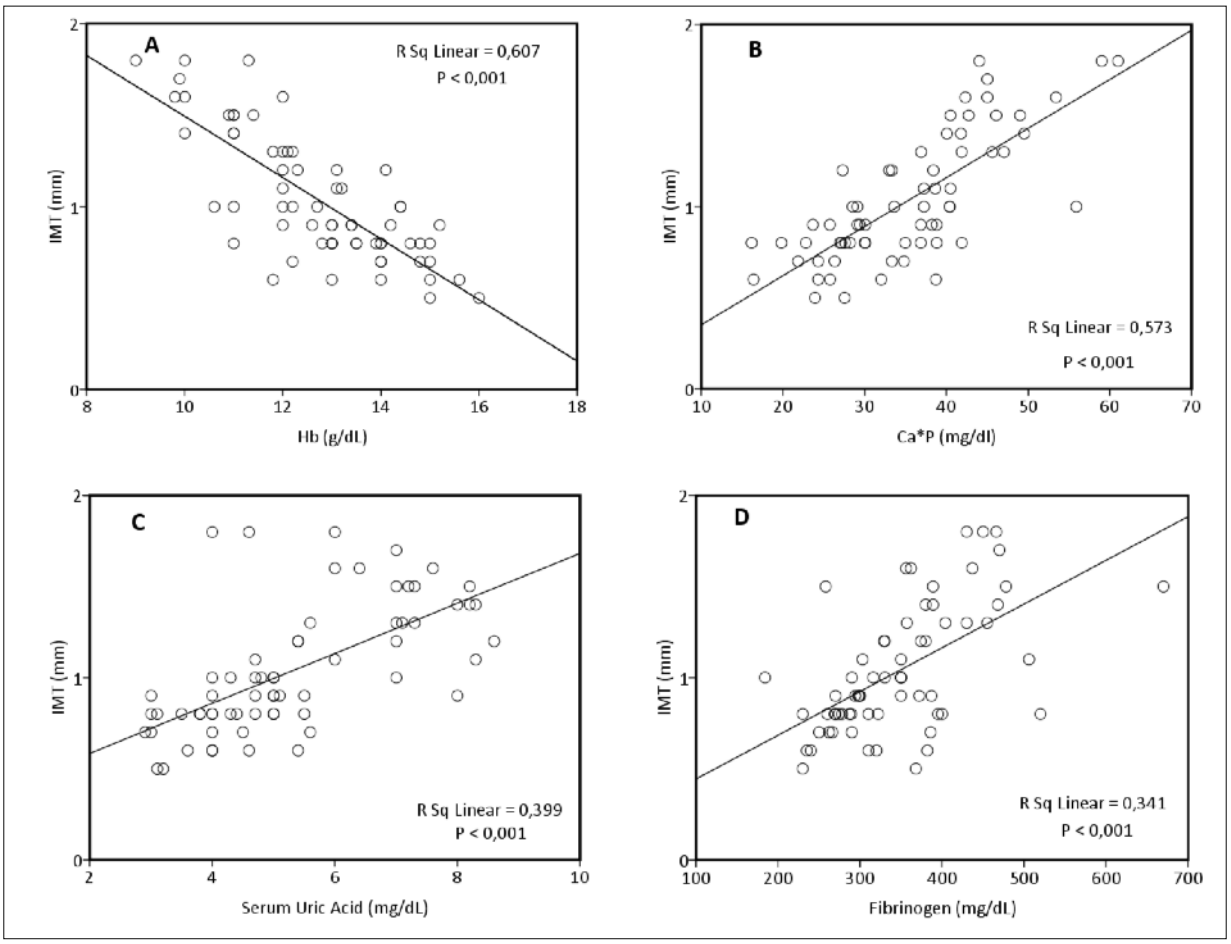

Figure 2. Linear regression plots of: ***(A)*** IMT vs serum Hb, r = –0.779, $p < 0.001$; ***(B)*** IMT vs serum Ca*P, r = 0.757, $p < 0.001$; ***(C)*** IMT vs Serum uric acid, r = 0.632, $p < 0.001$; ***(D)*** IMT vs Fibrinogen, r = –0.584, $p < 0.001$. *Abbreviations:* IMT, intima media thickness; Hb, hemoglobin; Ca*P, calcium X phosphate product.

Chronic flogosis appears to be primarily responsible for endothelial dysfunction with consequent accelerated atherosclerosis. In particular, the endothelium regulates the vascular tone through the balanced production of vasoconstrictive and vasodilative substances, such as NO[7]. Enhanced systemic inflammation is a common phenomenon in patients with kidney failure. Although the causes of inflammation are well-described in CKD, the downstream effects of inflammation on vascular function are not well elucidated. Our study showed an association between systemic inflammation and endothelial dysfunction in CKD patients, resulting in impaired vascular function and atherosclerosis. Already for some years[16], ultrasonographic study of postischemic vasodilatation of brachial artery has been proposed as the first-rate method for the noninvasive appraisal of endothelial function. Endothelial dysfunction is premature and systemic, and its vasodilatation (NO-mediated) alteration has been demonstrated in patients with documented atherosclerotic lesions and in those with cardiovascular risk factors[23-25]. Ultrasonographic evaluation of IMT is a valid and reproducible indicator of atherosclerotic disease[18]. An increased IMT in young and adult patients with cardiovascular risk factors is associated with systemic atherosclerosis, representing as an independent predictor factor of cardio/cerebrovascular events[26]. Furthermore, cIMT is useful to evaluate the effect of different treatments on the progression/regression of atherosclerosis. Thus, cIMT can be considered as an early index of generalized atherosclerosis, and its increase can precede, for many years, the development of atherosclerosis[27]. In our study, there was a progressive increase in inflammatory markers, uricemia, and homocysteinemia serum levels at Stage I and II CKD, along with progressive reduction in

brachial artery FMD and progressive increase in cIMT, which are both early and systemic markers of atherosclerotic disease and independent predictors of cardiac and cerebrovascular events[3-4] in patients with cardiovascular risk factors[23-25].

Conclusions

Our study, suggests that the serum levels of inflammatory parameters (PCR, fibrinogen, ferritin), uricemia, and homocysteinemia, as well as the ultrasonographic study (by estimating the reactivity and vascular wall alterations through FMD of brachial artery and cIMT) can be used as markers of early subclinical atherosclerosis in nephropathic patients with high cardiovascular risk factors. Although the ultrasonographic parameters appear to be feasible, widely reproducible, noninvasive, and well tolerated in a majority of patients, it is a little expensive with an interoperator variability. Moreover, brachial artery FMD, cIMT, and inflammatory markers can be useful to evaluate the efficacy of eventual therapeutic strategies by reducing endothelial damage (ACE inhibitors, statins, ATII antagonist, etc.)[26,27], and to correct and/or reduce the progressive factors of renal damage (e.g., arterial hypertension, proteinuria, hyperuricemia, hypercholesterolemia, hyperglycemia, etc.) as "primum movens" of chronic inflammatory state and following atherosclerosis disease[28,29]. Ultrasonographic parameters, FMD, and cIMT are becoming the first choice within noninvasive vascular diagnostic methodology for the evaluation of endothelial function in CKD patients with high cardiovascular risk factors. Uric acid and Hcy serum levels are noted to be inversely correlated to brachial artery FMD and positively correlated to cIMT, CRP, fibrinogen, and ferritin[30].

The elevated serum uric acid level, per se, may constitute a novel risk factor for endothelial dysfunction and may represent an important therapeutic target for mitigating cardiovascular risk in CKD[31]. Furthermore, this study suggests that elevated plasma Hcy level in patients with mild renal failure is associated with cIMT[32], which can be considered as early markers of endothelial dysfunction, inflammation, and atherosclerosis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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