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Proteoglycan/glycosaminoglycan and collagen content in the arterial wall of patients with end-stage renal disease - new indicators for vascular disease

Short title: Arterial wall PG/GAGs and collagen are new indicators in CKD

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What’s new?

Recently, the attention of researchers has focused on identifying novel predictive measures for chronic kidney disease and its remarkably high cardiovascular risk, which remains a leading cause of mortality in this demographic. Our study examines a particularly susceptible population of uremic and hemodialyzed patients. Prior reports indicate that extracellular matrix is a dynamically changing structural network, which is subject to alterations, i.e. in proteoglycan and collagen content, even in very early stages of vessel pathology. Through an array of biochemical (an extensive panel of established and emerging biomarkers) and morphological (semi-quantitative histology) assessments, we report on significant trends that are tied to the unique milieu of renal failure. We identify several potential predictors for the shift in matrix composition, which reflects a purported initial stage of developing vascular disease. Plasminogen-activator inhibitor is recognized as a molecule of particular interest. Our findings expand the current research for promising renovascular markers.
Abstract

**Introduction:** Cardiovascular (CV) comorbidity is high in chronic kidney disease, particularly in end-stage renal disease (ESRD). Novel biomarkers are of interest.

**Objectives:** Investigate relationships between matrix proteoglycans/glycosaminoglycans (PG/GAGs), collagen and calcifications in arteries with selected serum and plasma markers of endothelial dysfunction, inflammation, oxidative stress and bone turnover in patients with ESRD.

**Patients and methods:** 47 adult patients (32 male) with stage 5 chronic kidney disease were included. Fibrinogen, soluble thrombomodulin (sTM), plasminogen activator inhibitor-1 (PAI-1), stromal cell-derived factor-1 alpha (SDF-1α), calcium (Ca), phosphate (Pi), intact parathormone (iPTH), interleukin-6 (IL-6), highly sensitive C-reactive protein (hsCRP), ferric reducing ability of plasma (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, ferric reducing ability of ascorbate in plasma (FRASC), fetuin-A, fibroblast growth factor 23 (FGF-23), osteopontin (OPN), osteoprotegerin (OPG), osteocalcin (OC), transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), secreted protein acidic and rich in cysteine (SPARC), and matrix metalloproteinase 2 (MMP-2) were investigated. Radial artery specimens were stained with alizarin red for calcifications, alcian blue for PGs/GAGs and sirius red for collagen.

**Results:** PGs/GAGs, collagen, and calcification stainings were all positively inter-correlated. Most intense (grade 3) alcian blue staining was significantly associated with diabetes, higher Ca×Pi, hsCRP, fibrinogen, SDF-1α, PAI-1, and thrombomodulin. PAI-1 was the only significant predictor of grade 3 alcian blue in multiple logistic regression adjusted for hemodialysis, Ca×Pi and hsCRP.
Conclusions: Coagulative and endothelial dysfunction are hallmarks of ESRD. SDF-1α, PAI-1, thrombomodulin and fibrinogen may be novel risk indicators for early vascular wall alterations and serve as CV risk biomarkers.

Key words: biomarkers, end-stage renal disease, glycosaminoglycans, plasminogen activator inhibitor-1, proteoglycans

Introduction

The extracellular matrix (ECM) of vascular walls is a complex, polymeric structure composed of proteoglycans (PGs), elastin, collagens and a variety of glycoproteins acting to preserve vascular integrity and transduce signals[1]. PGs participate in collagen modification, growth factor retention, and chemokine signaling[2]. In vascular disease, ECM composition and regulation is altered, as illustrated by the accumulation of PGs and smooth muscle cells (SMCs) in early atherosclerotic lesions[1]. A role for ECM remodeling is increasingly recognized in calcification, which is associated with highly prevalent cardiovascular (CV) morbidity in chronic kidney disease (CKD)[3, 4], which itself has incited an ongoing search for biomarkers[5–7]. Genovese et al. recently proposed that ECM, as the underlying setting for fibrosis, should be investigated to identify biomarkers of ECM remodeling in renal disease[8].

Plasminogen activator inhibitor type-1 (PAI-1) is a molecule negatively regulating proteolytic activity of plasmin, which dissolves fibrin and activates matrix metalloproteinases, known regulators of ECM[9, 10]. Dysregulation of fibrinolysis may result from an enhanced level of PAI-1, which has been linked to cardiovascular disease[11], insulin resistance, metabolic syndrome and development of vascular bed abnormalities, including endothelial and coagulative dysfunction[9]. Congruently, these processes are well recognized in CKD pathogenesis. Indeed, hemostatic molecules, including serum thrombomodulin (sTM), an endothelial marker of injury, have been correlated with inflammation and surrogate markers of atherosclerosis[12]. Enhanced
PAI-1 production may also be involved in endothelial dysfunction and early atherogenesis[13]. A systematic meta-analysis in 2017 confirmed the association between PAI and coronary heart disease, independent of traditional CV risk factors[14]. Taken together, coagulative abnormalities seem to be closely interrelated with several processes shaping CV morbidity and may mediate known pathways of atherogenesis.

Chronic inflammation is another key player in CKD. In renal failure patients, elevated C-reactive protein (CRP) and diabetes were demonstrated as independent risk factors for CV events[15]. High CRP levels increase the odds for CV disease, independent of traditional risk factors[16]. Małyszko et al. indicated that adipose tissue may also contribute to CV disease by releasing inflammatory molecules such as TNFα, adiponectin and PAI[17]. The Chronic Renal Insufficiency Cohort recently reported that elevated TNFα and fibrinogen are independent predictors of CKD progression, and may be useful in risk stratification[18].

The aim of the present study was to determine the associations of several known and emerging biomarkers of inflammation, oxidative stress, endothelial dysfunction and bone turnover with changes in matrix composition and calcifications of radial arteries assessed morphologically in stage 5 CKD.

**Patients and Methods**

This study adopts a similar methodology to our earlier investigations[6, 19, 20], which were conducted as part of a concluded research initiative, designed by K.K., at the Department of Nephrology of the Jagiellonian University in Kraków. This cross-sectional investigation of the relationship between alcian blue and sirius red staining of arterial wall with vascular morphology
and an array of biochemical parameters is unique to this study and has not been published previously.

Consecutive patients with CKD at the Department of Nephrology of the Jagiellonian University in Krakow were screened for potential eligibility and recruited if they fulfilled the following inclusion criteria; stage 5 CKD and necessity to establish vascular access for hemodialysis via creation of arteriovenous fistula (AVF), which would enable sampling of radial artery fragments for further histological assessment. Patients were not included if any of the following were recorded by the study physician; active infection, positive HBV or HCV tests, HIV infection, renal transplantation, parathyroidectomy and neoplasm.

Forty-seven adult patients (15 women, 32 men) were included into this study, of which nineteen patients were in pre-dialysis stage, while 28 were already undergoing hemodialysis (HD) on a short-term or permanent catheter. Patient characteristics and clinical profile are shown in Table 1. Medical history was reviewed in detail; including information about comorbidities, diabetes mellitus, hypertension, dyslipidemia, current smoking status, duration of dialysis, and medication use. As part of a concluded research project, surgeons at our center collected specimens of radial artery tissue while establishing autologous AVF. Following this procedure, a pre-defined array of laboratory markers of inflammation, oxidative stress, endothelial dysfunction and bone turnover was assayed.

**Histology**

Samples of radial arteries were collected during AVF procedures. Staining with alizarin red for calcium deposits, with alcian blue to demonstrate matrix proteoglycans and glycosaminoglycans and with sirius red to identify collagen was followed by microscopic evaluation. Brightfield images were obtained using an Olympus DP-71 digital CCD camera with Olympus AnalySIS FIVE software. Blinded assessment was performed by an experienced
histologist. Degree of vascular calcification was semi-quantitatively evaluated according to the following scale: 0 - no mineral content, 1 - a few small dispersed concretions, 2 - numerous small dispersed concretions, 3 - larger granular concretions, 4 - large areas occupied by fused mineral deposits. Calcification was limited to the vascular media in all samples. The relative content of PG/GAG was also assessed semi-quantitatively (staining intensity grades 1-3).

Reproducibility of the adopted method of histological analysis has been documented and described elsewhere[19].

**Laboratory tests**

Sampling of serum and plasma from peripheral venous blood was performed in patients who underwent overnight fasting. Procedures were performed prior to the scheduled surgery for AVF creation. For plasma samples, blood was collected into ethylenediaminetetraacetic acid tubes and subsequently frozen at −70 °C and stored for further biochemical study.

In all patients, the following array of biochemical parameters was measured: creatinine, fibrinogen, soluble thrombomodulin (sTM), plasminogen activator inhibitor-1 (PAI-1), stromal cell-derived factor-1 alpha (SDF-1α), calcium, phosphate, intact parathormone (iPTH), interleukin-6 (IL-6), highly sensitive C-reactive protein (hsCRP), ferric reducing ability of plasma (FRAP), 2,2-diphenyl-1-picrylhydrazyl scavenging (DPPH), ferric reducing ability of ascorbate in plasma (FRASC), fibroblast growth factor 23 (FGF-23), osteopontin (OPN), osteoprotegerin (OPG), osteocalcin, transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), secreted protein acidic and rich in cysteine (SPARC), and matrix metalloproteinase 2 (MMP-2). Biochemical assays were assessed with the use of automatized analyzers: Hitachi 917 (Hitachi, Japan) and Modular P (Roche Diagnostics, Mannheim, Germany).
Markers of endothelial impairment, inflammation and mineral-bone disorder were investigated with enzyme-linked immunosorbent (ELISA) micro-plate assays through an ELX808 automatic reader (BIO-TEK® Instruments Inc., Vermont, VT, USA). The following kits were applied: OPG (BioVendor, Brno, Czech Republic); OPN, HGF, IL-6, TGF-β, SPARC and MMP-2 (R&D Systems, Minneapolis, USA); OC (Metra/Quidel, CA, USA), FGF-23 (Immunotopics Int., San Clemente, CA, USA). sTM levels in platelet-poor EDTA plasma samples were measured using commercially available ELISA (Human Thrombomodulin/BDCA-3 Immunoassay-R&D Systems, Minneapolis, USA). SDF1α levels in platelet-poor EDTA plasma samples were measured using commercially available ELISA (Human CXCL12/ SDF1α Immunoassay-R&D Systems, Minneapolis, MN, USA). PAI-1 concentration in platelet-poor EDTA plasma samples were measured using commercially available ELISA (Human Serpin E1/PAI-1 Immunoassay-R&D Systems, Minneapolis, MN, USA). The reference range is 0.99-16.9 ng/ml, mean – 5.1 ng/ml, standard deviation- 3.74 ng/ml.

Plasma was investigated for total antioxidant capacity, as determined by the ability of plasma to reduce Fe3+ to Fe2+ (ferric reducing ability of plasma), in accordance with Benzie’s method. DPPH radical scavenging assay was used to determine radical scavenging capacity of plasma, as described by Janaszewska. Ferric reducing ability of ascorbate in plasma was measured spectrophotometrically.[21, 22]

2.4. Statistical analysis

Quantitative data were reported as number (n) of patients and percentage of the group. Qualitative data were reported as median (lower-upper quartile). Correlations between ordinal variables were assessed using Goodman and Kruskal gamma coefficient. Contingency tables were analyzed with chi-squared test. Laboratory results were compared between the groups using
Mann-Whitney test or Kruskal-Wallis ANOVA. Logistic regression was used to assess predictors of high-grade histological staining (i.e. grade 3 alcian blue and grade 3 sirius red). The multiple logistic regression models were adjusted for HD status. The models included independent variables that were significantly associated with the outcome variable in simple analysis, however, the variables that were highly correlated (e.g. hsCRP and fibrinogen) were not included in a single model to avoid redundancy. The original qualitative variables were included in the models (they were not categorized) and the odds ratios were reported for 1 unit increase. Results were considered significant at p<0.05 in two-tailed tests. Statistica 12 (StatSoft, Tulsa, OK, USA) was used for computations.

Results

Clinical characteristics of patients

Basic clinical characteristics did not differ significantly between uremic (pre-dialysis) and HD patients. Hypertension and dyslipidemia were the most prevalent conditions, while approximately one fourth of patients were diabetics (Table 1). Conservative pre-dialysis and HD patients differed in their strategy of medication. Statins, antiplatelets and diuretics were more commonly used in pre-dialysis stages as compared to HD, while the reverse was observed for erythropoietin analogues (Table S1).

Histological examination of radial arteries

Radial artery sections were stained to identify matrix proteoglycans and glycosaminoglycans (alcian blue), collagen (sirius red) and calcifications (alizarin red). The distribution of staining grades among patients is shown in Table 2. Grades of the three stainings were significantly positively correlated, with gamma coefficients of 0.50 between alcian blue and
sirius red staining \((P=0.002; \text{ Figure 1A})\), 0.59 between alcian blue and alizarin red staining \((P<0.001; \text{ Figure 1B})\), and 0.38 between sirius red and alizarin red staining \((P=0.010; \text{ Figure 1C})\).

**Associations between histological staining and laboratory markers of hemostasis, endothelial dysfunction, bone turnover and inflammation**

Concentrations of the studied laboratory markers among uremic (pre-dialysis) and hemodialyzed patients are shown in Table 3. Dialyzed patients were characterized by higher concentrations of serum creatinine, calcium-phosphate product, FGF-23 (but not iPTH), OPN and sTM. Other studied laboratory markers did not differ significantly between the groups.

Grade 3 alcian blue staining was significantly associated with higher calcium-phosphate product, hsCRP, fibrinogen, SDF-1α, PAI-1, and sTM (Figure 2). PAI-1 was the only significant predictor of grade 3 alcian blue staining in multiple logistic regression adjusted for HD status, calcium-phosphate product and hsCRP concentrations (odds ratio 10.3; confidence interval 1.08-99.9; \(P=0.036\)). No significant associations were observed for sirius red staining. As we have reported earlier[6, 23], SDF-1α and sTM showed a positive correlation with alizarin red staining grade in patients with grade 1 to 3 staining \((P=0.002, P=0.028 \text{ respectively})\) (i.e. in the analysis excluding patients with grade 0 and grade 4 staining). However, patients with alizarin red staining of grade 4 showed relatively low concentrations of both these markers. We did not observe other associations between alizarin red staining grade and the concentrations of the studied laboratory markers.

**Associations between the intensity of histological staining with comorbidities and medical treatment**

We observed associations between the intensity of histological staining and diabetes. Patients with grade 3 alcian blue staining were more often affected by diabetes \((75\% \text{ vs. } 21\%; P=0.037)\), while calcifications detected by alizarin red (staining grade 1 to 4) were also more
often seen in diabetics (44% vs. 4.5%; \( P=0.002 \)). No such association was observed with sirius red staining for collagen. Alcian blue, sirius and alizarin red staining intensity was not associated with dialysis and smoking status, dyslipidemia, nor obesity. No statistically significant associations were observed between the use of medications listed in Table S1 and the results of alcian blue, sirius red and alizarin red stainings of radial artery samples. The use of statins was associated with higher concentrations of fibrinogen (Figure S1 – A), while the use of vitamin D was associated with higher concentrations of OPG, FGF-23 and HGF (Figure S1 – B-D). Moreover, the treatment with diuretics was associated with significantly lower osteopontin \(( P=0.011 \)), FGF-23 \(( P<0.001 \)) and IL-6 \(( P=0.003 \)).

**Discussion**

We report a significant association between calcium-phosphate product, hsCRP, fibrinogen, SDF-1\( \alpha \), thrombomodulin and PAI-1 with high grade alcian blue staining for matrix PGs and GAGs. These correlations with several “old” and “new” players involved in CKD and CV conditions may suggest their importance in early changes of the extracellular matrix and vascular wall structure. PAI-1 was the only significant predictor of grade 3 alcian blue staining in multiple logistic regression adjusted for dialysis status, calcium-phosphate product and hsCRP. Increased PAI-1 has previously been identified in severe atherosclerotic lesions and is thought to promote thrombosis\cite{24, 25}. Meanwhile, the relationship of PAI-1 in CKD vasculopathy and medial calcification has not been studied extensively. In models of arterial injury in atherosclerosis-prone, hyperlipidemic mice, PAI-1 knockout was shown to reduce neointimal formation and improve clearance of mural thrombi\cite{26}. Hertig et al. showed that PAI-1 deficiency lead to uncontrolled activation of TGF-\( \beta \), resulting in early murine glomerular
injury[27]. However, other authors report that TGF-β elevates PAI-1 expression, while its knockdown models exhibit decreased ECM deposition[28]. It seems that renovascular homeostasis requires a tight balance of fibrinolysis, to which abundance and shortage of PAI-1 can be detrimental. PAI-1 is thought to inhibit the modification of plasminogen and pro-metalloproteinases to active forms, preventing proteolysis of extracellular matrix[29]. Interestingly, Krag et al. observed that decreased ECM deposits following PAI-1 knockout were not associated with altered protease activity, which may relate to the difficulty in investigating the local microenvironment[28]. In the context of our findings, PAI-1 may serve as a marker for the accumulation of vascular PGs and GAGs, owing to its putative role in regulating matrix remodeling, which may occur independent of uremia, inflammation and calcium imbalance. Correlations with markers of inflammation, endothelial injury and coagulation may also reflect the offending nature of these processes in ESRD, rather than a shared pathomechanism.

Abundant GAGs in extracellular matrix of renal arteries have been described in ESRD and topographical proximity of intimal PGs and collagen was suggested to imply their close interaction[30]. Strauss et al. studied rabbit models of arterial injury and undamaged controls, where prominent early vascular changes were the several fold increase in extracellular matrix PGs, elastin and collagen, when intimal and medial proliferation was only modest[31]. PG components of the ECM partake in vascular cell migration and in vascular wall remodeling through retention and interaction with chemokines, growth factors and modifying collagen[2]. The present study demonstrates that matrix GAGs and PGs are positively correlated with collagen deposits and medial calcifications. Moreover, PGs have been described as abundant in early vascular lesions, coinciding with reduced collagen fibers, which then gradually increase in number with their progression[1]. Similarly, we observed that an increase in alcian blue staining
grade significantly coincides with a higher grade of Sirius red staining for collagen. Positive staining with alcian blue may further aid in identifying the early vascular lesions in CKD patients.

In the present study, the majority of radial artery specimens showed low to moderate grades of staining for matrix PGs and collagen. Calcifications were, overall, less advanced and present only in the media. Similarly, Ballantini et al. observed that light to moderately-advanced medial calcifications are the most prevalent in non-diabetic patients undergoing kidney transplantation, underscoring the importance of kidney disease itself[32]. However, we observed increased radial artery calcification levels in patients with higher content of PGs/GAGs and collagen. In a rabbit model of renal failure lasting 9 months, increased PGs and calcifications were predominant in the media, while intimal lesions were limited to increased cellularity. Calcifications were accompanied by accumulation of extracellular substance stained with alcian blue and of smooth muscle-like cells, with no evidence for inflammation or lipid retention[33]. Subsequent studies demonstrated that increased “alcianophilic” extracellular substance was one of the earliest vascular changes in chronic renal failure models[34]. Together, these findings suggest that changes in ECM can be useful in monitoring of early vascular remodeling in ESRD, when processes of inflammation and dyslipidemia are still subclinical.

We investigated whether increased PGs/GAGs content in the vascular wall revealed by the highest grade of alcian blue staining is correlated with selected clinical data, comorbidities and biomarkers of endothelial dysfunction, inflammation, oxidative stress and bone turnover. A generalized state of inflammation in CKD, phosphate, uremic toxins, glucose and imbalance of calcification inhibitors is theorized in the pathogenesis medial arterial calcification (MAC)[35]. Our results indicate a correlation between vascular matrix alterations and diabetes, while dialysis, smoking status, medications, dyslipidemia, and obesity did not reach significance. In a study
comparing diabetic and nondiabetic HD patients, calcifications were reported more frequently in diabetics and linked to poor glycemic control[36]. Our findings support the association of both PG/GAG accumulation and MAC with diabetes in ESRD, which may indicate shared and synergistic stimuli of injury. Indeed, Klein et al. showed that long lasting high glucose exposure increases PG synthesis[37]. GAGs in the arterial media are also altered throughout the course of diabetes, in a manner dissimilar of atherosclerosis[38]. However, the consequences of PG/GAG alterations in the vascular wall have not yet been fully elucidated. Disruption in the balance of versican and hyaluronan leads to impaired mechanical function and structural pro-proliferative changes to the intima[39]. High glucose level can alter perlecan and increase the number of monocytes in subendothelial matrix[40]. More recent studies attribute a role in aortic calcification to glycosaminoglycans[41], linked to bone morphogenetic protein-2 signaling under high phosphate conditions. In light of these findings, we postulate that a shift in the balance of extracellular matrix PGs/GAGs may be involved in diabetic vascular complications of ESRD patients.

In this study we provide evidence that increased PG/GAG content in the vascular wall shares a relationship with medial calcifications. This adds on to our earlier findings of the relationship between MAC with SDF-1 and sTM[6, 23], the latter two also positively correlated with matrix alterations (high grade alcian blue staining). TM is an endothelial surface lining proteoglycan with a potent anticoagulative function, shed into a soluble form in ESRD[42, 43]. In HD patients, elevated sTM is thought to reflect endothelial injury and is improved after kidney transplantation[44]. Widely prevalent, recurrent endothelial injury may lead to mobilization of endothelial progenitor cells from bone marrow, to rebuild the damaged vascular barrier in ESRD[45, 46]. Importantly, this response seems to be severely impaired in CKD milieu. SDF-1, together with other angiogenic factors, has been implicated in migration of EPCs[46]. Drawing
the associations between biomarkers of relevant processes, we postulate that as one of the events culminating in vascular disease, matrix alterations may occur following chronic endothelial injury, inflammation and altered mineral imbalance. Our findings may uniquely translate into clinical application. However, this relies on the putative relationship of biochemical markers, ECM turnover, and vasculopathy, which could be indirectly followed by simple staining procedures in patients undergoing vascular access surgeries. Our findings serve as an evidence benchmark, and preliminarily establish the usefulness of alcian blue staining, with its predictors of fibrinogen, PAI-1, sTM and SDF1α. They may warrant further longitudinal study with hard outcomes not only in ESRD, but also in other related, vasculature-involving conditions.

Although uremic and HD patients differed in drug usage, none of the medications were associated with staining for PG/GAG, collagen, nor calcification. However, this did not remain true for all biomarkers, for some of which we observed several correlations with chosen therapy. These relationships remain difficult to interpret without individual clinical context, as standalone, and are therefore outside the scope of this paper.

Limitations

An inherent limitation of present study is present in the cross-sectional character, which confines the hypotheses drawn. Causality cannot be determined in this exploratory design, by a comparison with literature and statistical analysis alone. We discuss the purported relationship between mediators, which may only be markers of processes co-occurring in renal failure, without any direct mechanistic link. Our morphological analysis can also be criticized as crude when considering the complexity of alterations in local vascular microenvironments. Notwithstanding these substantial limitations, our study serves in a unique attempt to translate experimental research of ECM turnover and CKD mediators/biomarkers into an inpatient setting for ESRD. Cost-efficiency of imaging diagnostic procedures, such as coronary artery
calcification score, might be limited due to financial strain when frequently screening at first dialysis. Our results are promising of the view that a validated panel of CV risk biomarkers for CKD patients may be developed in the future.

**Conclusions**

We postulate that SDF-1α, PAI-1, thrombomodulin and fibrinogen may be novel risk indicators for early changes in the arterial wall. They may be involved with very early processes of vessel remodeling and calcification in patients with advanced kidney disease and can be useful, simple biomarkers of CV risk in daily practice.

**Disclosure Statement**

The manuscript has not been published elsewhere.

**Conflict of Interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.

**Acknowledgments**

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**Contribution statement:**

KK, KB conceived the study, were the major participants in its design, coordination, interpretation of results and statistical analysis, they also prepared draft manuscript. MG carried out histological examinations. PD performed statistical analysis and drafted the results. MK, AP,
DF, KW participated in the design of the study, interpretation of results and statistical analysis. PG participated in design of the study and analyzed the data. JAL participated in data analysis and in preparation of the final manuscript version. MAK and WS participated in study design and coordination. All authors were involved in data collection, draft manuscript modifications and approved the final version of the manuscript.

References


[34] Tvedegaard E. Arterial disease in chronic renal failure--an experimental study in the


Table 1. Demographic and clinical characteristics of the studied stage 5 CKD patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pre-dialysis patients (n=19)</th>
<th>Patients on hemodialysis (n=28)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>62 (56; 72)*</td>
<td>60 (48; 74)</td>
<td>0.5</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>13 (68)</td>
<td>19 (68)</td>
<td>0.9</td>
</tr>
<tr>
<td>Maintenance hemodialysis duration, months</td>
<td>NA</td>
<td>5.5 (1.0; 27.0)</td>
<td>NA</td>
</tr>
<tr>
<td>eGFR (MDRD) among non-dialized, ml/min/1.73 m²</td>
<td>13 (10; 14)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.1 (23.6; 30.9)</td>
<td>24.6 (21.9; 28.1)</td>
<td>0.08</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>6 (32)</td>
<td>6 (21)</td>
<td>0.4</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>18 (95)</td>
<td>22 (79)</td>
<td>0.2</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>12 (63)</td>
<td>14 (50)</td>
<td>0.5</td>
</tr>
<tr>
<td>Active smoking, n (%)</td>
<td>4 (21)</td>
<td>10 (36)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*Quantitative data are reported as median (lower quartile; upper quartile).

Statistically significant (P<0.05) differences are provided in bold.

Abbreviations: Body mass index, BMI; estimated glomerular filtration rate, eGFR; modification of diet in renal disease, MDRD; NA, not applicable
Table 2. Semi-quantitative assessment of radial artery content of matrix proteoglycans/glycosaminoglycans, collagen and calcifications in patients with stage 5 CKD

<table>
<thead>
<tr>
<th>Staining</th>
<th>Results among stage 5. CKD patients (n=47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radial artery matrix proteoglycans and glycosaminoglycans (alcian blue staining):</td>
<td></td>
</tr>
<tr>
<td>grade 1, n (%)</td>
<td>16 (34)</td>
</tr>
<tr>
<td>grade 2, n (%)</td>
<td>27 (57)</td>
</tr>
<tr>
<td>grade 3, n (%)</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Radial artery collagen (sirius red staining):</td>
<td></td>
</tr>
<tr>
<td>grade 1, n (%)</td>
<td>10 (21)</td>
</tr>
<tr>
<td>grade 2, n (%)</td>
<td>24 (51)</td>
</tr>
<tr>
<td>grade 3, n (%)</td>
<td>12 (26)</td>
</tr>
<tr>
<td>no data, n (%)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Radial artery calcification (alizarin red staining):</td>
<td></td>
</tr>
<tr>
<td>grade 0, n (%)</td>
<td>22 (47)</td>
</tr>
<tr>
<td>grade 1, n (%)</td>
<td>12 (26)</td>
</tr>
<tr>
<td>grade 2, n (%)</td>
<td>4 (9)</td>
</tr>
<tr>
<td>grade 3, n (%)</td>
<td>4 (9)</td>
</tr>
<tr>
<td>grade 4, n (%)</td>
<td>5 (11)</td>
</tr>
<tr>
<td>Laboratory test</td>
<td>Predialysis patients (n=19)</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Creatinine, μmol/l</td>
<td>396 (258; 452)</td>
</tr>
<tr>
<td>Fibrinogen, g/l</td>
<td>5.12 (4.34; 5.52)</td>
</tr>
<tr>
<td>hsCRP, mg/l</td>
<td>3.28 (2.14; 6.91)</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>2.94 (2.15; 4.18)</td>
</tr>
<tr>
<td>TGF-β1, ng/ml</td>
<td>6.44 (6.09; 8.63)</td>
</tr>
<tr>
<td>HGF, ng/ml</td>
<td>1.80 (1.56; 2.37)</td>
</tr>
<tr>
<td>PAI-1, ng/ml</td>
<td>1.54 (1.01; 2.35)</td>
</tr>
<tr>
<td>sTM, ng/ml</td>
<td>16.4 (12.8; 17.6)</td>
</tr>
<tr>
<td>SDF-1α, pg/ml</td>
<td>2894 (2666; 3155)</td>
</tr>
<tr>
<td>Ca x P, mmol²/l²</td>
<td>2.98 (2.91; 3.25)</td>
</tr>
<tr>
<td>iPTH, pg/ml</td>
<td>306 (204; 457)</td>
</tr>
<tr>
<td>FGF-23, RU/ml</td>
<td>487 (398; 877)</td>
</tr>
<tr>
<td>OPN, ng/ml</td>
<td>217 (207; 320)</td>
</tr>
<tr>
<td>OPG, pmol/l</td>
<td>5.12 (2.81; 6.96)</td>
</tr>
<tr>
<td>OC, mg/ml</td>
<td>41.7 (27.1; 54.1)</td>
</tr>
<tr>
<td>SPARC, ng/ml</td>
<td>108 (74; 159)</td>
</tr>
<tr>
<td>MMP-2 ng/ml</td>
<td>214 (191; 270)</td>
</tr>
<tr>
<td>FRAP, mmol/l</td>
<td>1.04 (0.64; 1.31)</td>
</tr>
<tr>
<td></td>
<td>FRASC, μmol/l</td>
</tr>
<tr>
<td>---------------------</td>
<td>--------------</td>
</tr>
<tr>
<td></td>
<td>49.0 (36.2; 57.3)</td>
</tr>
<tr>
<td></td>
<td>50.2 (43.8; 58.8)</td>
</tr>
</tbody>
</table>

Data are reported as median (lower-upper quartile).

Statistically significant ($P<0.05$) differences are provided in bold.

Abbreviations: calcium, Ca; 2,2-diphenyl-1-picrylhydrazyl scavenging, DPPH; ferric reducing ability of ascorbate in plasma, FRASC; ferric reducing ability of plasma, FRAP; fibroblast growth factor 23, FGF23; hepatocyte growth factor, HGF; highly sensitive C-reactive protein, hsCRP; intact parathormone, iPTH; interleukin-6, IL-6; matrix metalloproteinase-2, MMP2; osteocalcin, OC; osteopontin, OPN; osteoprotegerin, OPG; plasminogen activator inhibitor-1, PAI-1; phosphate, Pi; secreted protein acidic and rich in cysteine, SPARC; soluble thrombomodulin, sTM; stromal cell-derived factor-1 alpha, SDF-1α; transforming growth factor-β, TGFβ
Figure 1. Interrelations between grades of histological staining for matrix proteoglycans and glycosaminoglycans (alcian blue) and collagen (sirius red) (Figure 1A), and calcification (alizarin red) (Figure 1B); the associations between grades of histological staining for collagen (sirius red) and calcification (alizarin red) (Figure 1C)
Figure 2. The associations between CaxP index and alcian blue staining grade (Figure 2A); serum CRP concentrations and alcian blue staining grade (Figure 2B); serum CRP concentrations
and alcian blue staining grade (Figure 2B); serum fibrinogen concentration and alcian blue staining grade (Figure 2C); serum SDF1α concentrations and alcian blue staining grade (Figure 2D); serum thrombomodulin concentrations and alcian blue staining grade (Figure 2E); serum PAI-1 concentrations and alcian blue staining grade (Figure 2F).

Data are shown as median, interquartile range (box), non-outlier range (whiskers), and outliers (dots).