Aim: Cardiovascular disease is the leading cause of morbidity and mortality among patients on dialysis. The aim of this study was to identify the relationship between cardiovascular disease and arterial stiffness in patients on renal replacement therapy.

Patients-Methods: We studied 96 patients (34 on conventional haemodialysis, 42 on haemodiafiltration and 20 on peritoneal dialysis). On enrollment arterial stiffness was evaluated by measuring the carotid-femoral pulse wave velocity (c-f PWV). We also measured beta2-microglobulin (beta2M) and high sensitivity C-reactive protein (hsCRP) levels as well as Kt/V for urea. Cardiovascular disease was defined as the presence of coronary artery disease (CAD), heart failure (HF) and peripheral vascular disease (PVD).

Results: After adjustment for traditional risk factors c-f PWV and Kt/V for urea were independent predictors of CAD (B=0.307, OR=1.359, 95.0% CI 1.115-1.657, p=0.002 and B=0.838, OR=2.312, 95.0% CI 1.077-4.964, p=0.032 respectively). HF could be predicted independently by c-fPWV (B=0.244, OR=1.277, 95.0% CI 1.028-1.585, p=0.027). We also observed a positive correlation between hsCRP and both, beta2M levels and c-fPWV (r=0.257, p=0.01 and r=0.239, p=0.021 respectively).

Conclusions: Arterial stiffness is an independent predictor of coronary artery disease and heart failure in patients on renal replacement therapy, hsCRP is associated with c-fPWV and beta2-microglobulin levels indicating that inflammation may contribute the development of arterial stiffness and accelerated atherosclerosis in these patients.

Key words: arterial stiffness, beta2-microglobulin, cardiovascular disease, haemodialysis, inflammation, peritoneal dialysis.

Introduction

Cardiovascular disease is the leading cause of morbidity and mortality among patients on dialysis, and this may be partially explained by accumulation of risk factors for atherosclerosis in this population.

The majority of deaths are related to vascular disease including myocardial infarction, cerebrovascular strokes and peripheral ischemic events. Although coronary atherosclerosis is a well-known
underlying cause of cardiovascular disease, arterial alterations include more widespread vascular changes, that contribute to stiffening of the arteries. Arterial hardening results in increased pulse wave velocity (PWV) and pulse pressure (PP)\(^2\).

The aim of this study was to investigate the relationship between the cardiovascular disease and arterial stiffness in patients on renal replacement therapy.

**Patients-Methods**

**Patients**

Ninety-six dialysis patients (62 male), were enrolled who provided informed consent prior to participation. Mean age of the patients was 62.1±14.3 years. The treatment modalities which were applied were: conventional haemodialysis (HD, n=34), online-predilution haemodiafiltration (OL-HDF, n=42) and peritoneal dialysis (PD, n=20). The median time on haemodialysis was 5.0 years (range 3-10 years) and the mean time on peritoneal dialysis was 2.8±1.6 years.

The enrolled patients were in a steady state without serious clinical events for three months prior to enrollment. Laboratory and haemodynamic parameters were recorded at baseline. Exclusion criteria were the existence of acute illness or infection and malignancy. We also excluded patients with multiple intradialytic hypotensive episodes, interdialytic orthostatic hypotension, atrial fibrillation, uncontrolled hypertension and patients with an interdialytic weight gain of >5% of total body weight. The enrolled patients did not have interdialytic peripheral oedema or other characteristics of poor dry body weight assessment.

Finally, patients were excluded if they had Kt/V for urea <1.2. Patients were considered hypertensive if they had pre-dialysis blood pressure >140/90 mmHg, or if they were receiving anti-hypertensive drugs. Hypertension was controlled with calcium channel blockers in all patients.

Only calcium-free phosphate binders were prescribed.

All of the enrolled patients were on erythropoetin-a or-β therapy.

The following risk factors of atherosclerosis were examined: age, dialysis duration, history of smoking obtained by a self-administered questionnaire (n=21, 21.9%), existence of left ventricular hypertrophy (LVH, n=54, 56.3%), lipid profiles [total cholesterol, triglycerides, high and low density lipoprotein (HDL and LDL respectively)], diabetes mellitus (n=11, 11.5%) and peripheral vascular disease (PVD, n=39, 40.6%).

Clinical manifestations of atherosclerosis were defined as presence of coronary artery disease (CAD, n=30, 31.3%), and heart failure with an ejection fraction <50% (HF, n=26, 27.1%). The coronary syndrome was documented by history of myocardial infarction, coronary artery angioplasty or bypass surgery, or clinical signs of angina pectoris.

The haemodialysis treatment was performed 3-times weekly with a dialysis time of 3.5-4 h per session, and a blood flow rate of 550-400 ml/min. A bicarbonate-based ultrapure buffer dialysis solution was used with a dialysate flow rate of 550-600 ml/min, a calcium concentration of 1.50-1.75 mmol/L and a sodium concentration of 138-145 mmol/L. We used exclusively low-molecular weight heparin as an anticoagulant and synthetic high-flux dialysers of 1.5-2 m\(^2\) surface area, and an ultrafiltration coefficient >20 ml/h\(^3\).

Dialysis dose defined by Kt/V for urea was calculated according to Daugirdas formula\(^4\).

The enrolled peritoneal dialysis patients were on continuous ambulatory peritoneal dialysis (CAPD) with 4 exchanges per day using a combination of 2 exchanges of 2 L of hypertonic glucose-based solution (3.86% glucose; Baxter Healthcare) and 2 changes of 2 L of semi-hypertonic glucose solution (2.5% glucose; Ariti; Bieffe Medital S.p.A.).

Twenty haemodialyzed patients and 15 peritoneal dialyzed patients had 100 ml urinary output per day.

This study was approved by the ethics committee of the hospital.

**Laboratory measurements**

In haemodialyzed patients blood was drawn from the vascular access just before the start of a midweek dialysis session in a fasting state. In the end of the treatment flow rate blood was reduced to <80 ml/min and blood samples were obtained from the arterial dialysis tubing. In peritoneal dialyzed patients blood samples were also taken in a fasting state.

The concentrations of beta2M were measured by radioimmunoassay (Immunotech by Beckman, Czech Republic) with a reported inter-assay coefficient of variation ≤7.5%.

Albumin, cholesterol, triglyceride levels were measured by routine methods and LDL levels were calculated from the Friedewald equation\(^5\).
For the haemodialyzed patients, sodium (Na\(^+\)) levels in the start and in the end of the hemodialysis session were measured and sodium removal was determined as percent sodium removal (PSR) using the following formula: \((\text{Na}\(^+\)\text{pre} – \text{Na}\(^+\)\text{post} / \text{Na}\(^+\)\text{pre}) \times 100\).

In peritoneal dialysis patients, peritoneal equilibrium test (PET) was done after a four-hour dwell and 15 patients were found to be high or high average transporters, while 5 patients were low or low average transporters. The weekly clearance of urea (Kt/V/week) and creatinine (Ccr/week/1.73m\(^2\)) were calculated. Blood samples were taken at the start (time 0) and at the end of the four-hour dwell. The sodium removal (Na\(_{\text{rem}}\)) in these patients was calculated according to the following equation: \(\text{Na}_{\text{rem}} = \text{C}_{\text{Na, end}} \times \text{V}_{\text{D, end}} - \text{M}_{\text{Na}}(0)\), where \(\text{M}_{\text{Na}}(0)\) was the infused quantity sodium in the dialysate, \(\text{C}_{\text{Na, end}}\) was mean dialysate sodium concentration at 4 hours, and \(\text{V}_{\text{D, end}}\) was mean dialysate volume at 4 hours.

Normalized protein catabolic rate for dry body mass (nPCR) was calculated from the urea generation rate. Body mass index (BMI) was obtained from height and postdialysis body weight.

High sensitivity C-reactive protein (hsCRP) levels were measured using enzyme linked immunosorbent assay (ELISA, Immundiagnostik AG, Germany) with a reported inter-assay coefficient of variation ≤11.6%.

Haemodynamic measurements

Predialysis peripheral systolic and diastolic blood pressures (SBP and DBP respectively) in haemodialysis patients were calculated as the mean of 10 measurement during a treatment month using an automatic sphygmomanometer OMRON M4-I (Co Ltd Kyoto Japan). Mean peripheral pre-dialysis BP (MBP) was calculated as: \((\text{systolic BP} + \text{diastolic BP})/2\).

A 12-lead electrocardiographic examination was used to estimate the existence of left ventricular hypertrophy according to the recommendations of the American Society of Echocardiography.

Before a mid-week dialysis session, the patients on haemodialysis and the patients on peritoneal dialysis during their regular appointment into our Peritoneal Dialysis Unit, were allowed to rest for at least 10 minutes prior to their haemodynamic measurements.

Arterial stiffness was measured as carotid-femoral pulse wave velocity (c-fPWV) and carotid augmentation index (AIX) using the SphygmoCor system® (AtCor Medical Pty.Ltd, Sydney, Australia). In each subject two sequences of measurements were performed, and their mean was used for statistical analysis.

Central systolic blood pressure (cSBP), diastolic blood pressure (cDBP), mean blood pressure (cMBP), pulse pressure (PP), and the time of return of the reflected wave (Tr) were derived. Pressure and time of first peak (P1 and T1 respectively) and second peak (P2 and T2 respectively), and central augmented pressure (AP) were obtained. Central augmentation index (AIX) was computed (\(\text{AP} = \text{P2-P1}; \text{AIX} = (\text{AP}/\text{PP}) \times 100\)) and corrected for a heart rate of 75 beats/min.

Additionally, we measured the systolic pressure in both sides of the lower extremities, using a Doppler machine (Huntleigh Healthcare Ltd, UK). Ankle-brachial blood pressure index (ABPI) was calculated as the ratio of the lower values of ankle systolic pressure (pre or posttibial artery), divided by stabilized arm systolic pressure in haemodialysis and peritoneal dialysis patients. ABPI values <0.9 were rated as low indicating peripheral vascular disease (PVD) and values up to 1.2 were rated as high.

Statistical analysis

Normally distributed values were expressed as mean ± SD. Differences between mean values were assessed by unpaired \(t\)-test for two groups and one-way ANOVA analysis for three groups. Data that showed skewed distributions were expressed as median value ± interquartile range and were compared with Krussall-Willis test and Mann-Whitney U-test. Correlations between variables were assessed by Spearman coefficient.

We performed a Cox-regression analysis using forward stepwise method to predict the presence of CAD and HF. We investigated the dialysis modality, c-fPWV and LDL as possible independent factors, after adjustment for the traditional cardiovascular risk factors, such as age, sex, hypertension, smoking and diabetes mellitus. Additional factors which were entered into the analysis were: dialysis duration, urine output and treatment adequacy defined by Kt/V for urea.

Variables that were found to have a statistical association \((p<0.05)\) in univariate linear regression analysis were entered in the multivariate models and collinearity was tested estimating the condition indices and variance inflation factors (VIF).
Analysis was done using SPSS© v19.0 statistical software.

A p value <0.05 was considered as statistically significant.

Results

Clinical characteristics and laboratory parameters of the study population at baseline are presented in Table 1.

Predictors of cardiovascular disease Cox-regression analysis revealed that c-f PWV and dialysis adequacy were independent predictors of CAD (B=0.307, OR=1.359, 95.0% CI 1.115-1.657, p=0.002 and B=0.838, OR=2.312, 95.0% CI 1.077-4.964, p=0.032 respectively), after adjustment for dialysis modality and age, sex, hypertension, diabetes, smoking, dialysis duration, urine output and LDL serum levels.

In addition, HF could be predicted independently by c-fPWV (B=0.244, OR=1.277, 95.0% CI 1.028-1.585, p=0.027), after adjustment for the same above confounders.

Table 1. Clinical characteristics and haemodynamic and laboratory parameters in 96 dialysis patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.1</td>
<td>14.3</td>
</tr>
<tr>
<td>Dialysis duration (years)</td>
<td>6.3</td>
<td>5.7</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>25.084</td>
<td>3.8652</td>
</tr>
<tr>
<td>Urine (ml /day)</td>
<td>242.5</td>
<td></td>
</tr>
<tr>
<td>KTVurea</td>
<td>1.57</td>
<td>45</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.9</td>
<td>4.5</td>
</tr>
<tr>
<td>beta2-microglobulin (mg/L)</td>
<td>32.88</td>
<td>27.95</td>
</tr>
<tr>
<td>Total Cholest erol (mg/dl)</td>
<td>163</td>
<td>45</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>172</td>
<td>87</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>38.9</td>
<td>9.7</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>90.5</td>
<td>37.2</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>8.65</td>
<td>5.96</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>131.7</td>
<td>23.8</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80.9</td>
<td>10.5</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>106.3</td>
<td>16.2</td>
</tr>
<tr>
<td>Pulse Pressure (mmHg)</td>
<td>57.2</td>
<td>20.5</td>
</tr>
<tr>
<td>ABPI</td>
<td>1.09</td>
<td>4.9</td>
</tr>
<tr>
<td>c-f PWV (m/s)</td>
<td>11.30</td>
<td>1.87</td>
</tr>
<tr>
<td>Na removal in HD (PSR, %)</td>
<td>2.26</td>
<td></td>
</tr>
<tr>
<td>Na removal in PD (mmol/day)</td>
<td>184.27</td>
<td>41.08</td>
</tr>
</tbody>
</table>

Data are presented as mean ±SD.

Cardiovascular disease and dialysis modality

Comparing the groups of patients with and without CAD (n=30 vs n=66), significant differences were noted for age, PP and c-fPWV (p=0.02, p=0.001 and p=0.001 respectively) with a prevalence of CAD group. (Table 2)

Compared to patients without HF (N=70), patients with HF (n=26) presented higher PP, older age, higher c-fPWV and higher hsCRP serum levels (p=0.03, p=0.005, p=0.018 and p=0.001 respectively) (Table 2).

Also, compared to patients without PVD (n=57), patients with PVD (n=39) had higher PP, higher hsCRP and higher c-fPWV (p=0.05, p=0.017 and p=0.023 respectively) (Table 2).

Comparing the dialysis modalities, we noted that patients on HD presented significantly higher PP values, than patients on HDF and on PD (p=0.001 and p=0.04 respectively). In addition, patients on HD presented a trend for higher beta2M levels (p=0.06) than the patients on HDF. Dialysis duration was longer in patients on HDF than in patients on HD and on PD (p=0.001 and p<0.001 respectively). The values of ABPI did not differ between the three dialysis modalities.

Correlations of c-fPWV and PP

We observed a significant positive correlation between c-fPWV and age, PP and hsCRP (r=0.410, p=0.001, r=0.508, p=0.001 and r=0.239, p=0.021 respectively). After selecting only the patients on haemodialysis (n=76), we also observed that the correlation between c-fPWV and hsCRP was particularly significant (r=0.400, p=0.001) (Figure 1).

![Fig. 1. Correlation between hsCRP serum levels and c-f PWV in 76 haemodialysis patients.](image-url)
There were no significant interaction between age, PP and hsCRP in multivariate analysis.

On the other hand, PP was inversely associated with sodium removal and positively with age (r= -0.209, p=0.041 and r=0.350, p=0.001 respectively). We also observed a positive correlation between hsCRP and pre-dialysis beta2M levels (r=0.257, p=0.01).

**Discussion**

Cardiovascular disease in patients with end-stage renal disease is characterized by several cardiac and arterial disorders. Complications, as CAD, HF, stroke and peripheral vascular disease, represent the major causes of morbidity and mortality in these patients. The underlying pathophysiology that leads to the above events includes various mechanisms such as LVH, plaque formation, arterial stiffening, endothelial dysfunction and inflammation.11-13.

In this cross-sectional study, the presence of CAD and HF were predicted by c-fPWV independently of other traditional risk factors including age, sex, hypertension, diabetes, smoking habits and LDL, as well as uremia-related risk factors (such us dialysis duration, urine output or Kt/V for urea).

It is known that the prevention of cardiovascular disease focuses on the modifiable risk factors (dyslipidemia, hypertension, smoking), but also on evaluation of individual risk.14.

It has been previously reported that pulse wave velocity is an integrated index of vascular function and structure. It estimates arterial stiffness, which is a strong predictor of cardiovascular mortality both in the general population and in dialysis patients.13,14. The most frequent cause of cardiovascular death in dialyzed patients is congestive heart failure and sudden death. These two conditions are more likely to result from arteriosclerosis and arterial hardening.11. The above are in agreement of the independent correlation between c-fPWV and heart failure.

Additionally, pulse wave velocity is a major determinant of systolic hypertension, increasing left ventricular afterload, left ventricular hypertrophy and left ventricular oxygen consumption. Another consequence of arterial stiffening is the decreased diastolic blood pressure, which is associated with altered timing of wave reflection and decreased coronary perfusion contributing to ischaemic heart disease.15. These mechanisms explain our finding of coronary disease prediction by c-fPWV. Also, the decreased diastolic blood pressure in combination with the systolic hypertension result in increased pulse pressure. Indeed, according to our findings, patients with coronary artery disease were older and had higher pulse pressure and c-fPWV. Similarly, patients with heart failure were older age and had higher pulse pressure, and c-fPWV than patients without heart failure.

Moreover, in the present study, we noted that the dialysis adequacy was a significant predictor of CAD in combination with c-fPWV.
Previous studies showed that patient outcome improved with increasing fractional urea removal\textsuperscript{16}. However, HEMO Study showed that outcomes are not improved by increasing fractional urea removal above the current standard (Kt/V 1.05 into Kt/V 1.45)\textsuperscript{17}.

Nevertheless, treatment that removes the majority of urea should also remove more toxic solutes, like urea, that diffuse relatively freely. On the other hand, dialysis adequacy is ideally reported to clearance of middle molecular weight uremic toxins, such as beta2-microglobulin, along with small solutes, and, electrolyte and acid-base control, correction of anemia, optimization of nutritional status and improvement of quality of life. Convective therapies may offer significant benefits compared to diffusive therapies. However in this study, we did not observe any significant difference of Kt/V between hemodialysis modalities, but we observed a trend for significantly higher beta2M levels and pulse pressure (PP) in patients on conventional dialysis compared to patients on haemodialfiltration.

It should be noted that, a previous study, showed that increased PP may be mainly a consequence of fluid overload, rather than of increased arterial stiffness\textsuperscript{18}, despite the fact that the former can be associated with increased arterial stiffness\textsuperscript{19}.

However, in the present study, we observed that age, PP, and hsCRP were significantly correlated with c-fPWV, whereas co-linearity test did not show significant multico-linearity between the above parameters, suggesting an independent association between c-fPWV and these factors.

Also, in this study, and in agreement with another previous study\textsuperscript{20}, we observed a positive correlation between c-fPWV and CRP. It is well documented that atherosclerosis is an established inflammatory disease\textsuperscript{21} characterized by plaque and occlusive lesions formation responsible for coronary and peripheral artery disease\textsuperscript{11}.

PVD is an atherosclerotic disease, that affects mainly the lower extremities and it reduces functional capacity and quality of life\textsuperscript{22}. Also, PVD is frequently associated with coronary and cerebrovascular disease and patients with PVD are at increased risk for myocardial infarction and vascular death. In this study, we observed that patients with HF and patients with PVD presented significantly higher c-fPWV and hsCRP levels than patients without these diseases.

Additionally, we observed a significant positive correlation between hsCRP and pre-dialysis beta2M serum levels. In agreement with our finding, a recent study in haemodialysis patients\textsuperscript{23} showed that beta2M was significantly correlated with markers of inflammation (IL-6, TNF-\textalpha, hsCRP).

Beta2-microglobulin serves as a surrogate marker of other middle-molecular weight uremic toxins, that have been recognized as pathogenic substances implicated in the genesis of accelerated atherosclerosis in haemodialysis patients\textsuperscript{24,25}. In accordance with the above, beta2M was found to be a significant predictor of morbidity and mortality in uraemic patients independent of dialysis duration, diabetes, and malnutrition\textsuperscript{26}. Predialysis beta2M serum levels may provide dual information both, on dialysis efficacy and on internal bioactivity\textsuperscript{27}. It has been suggested that high concentrations of beta2M may induce apoptosis or necrosis in normal cells and the release of enzymes that act as chemoattractants for mononuclear cells resulting in initiation of the inflammatory response\textsuperscript{28}.

In conclusion, in patients on renal replacement therapy c-fPWV, is as an independent predictor of coronary artery disease and heart failure. Inflammation, is positively associated with beta2-microglobulin levels, and contributes to arterial stiffness and accelerated atherosclerosis in these patients.

**Περίληψη**

Αρτηριακή σκληρία και καρδιαγγειακή νόσος σε ασθενείς σε θεραπεία υποκατάστασης της νεφρικής λειτουργίας. B. Ράϊκου\textsuperscript{1}, A. Κυριάκη\textsuperscript{2}, N. Καταλάμπρος\textsuperscript{1}.

1 Α’ Παθολογική Κλινική, Εθνικό Καποδιστριακό Πανεπιστήμιο Αθηνών, 2 Τμήμα Πυρηνικής Ιατρικής, Γενικό Νοσοκομείο «Λαϊκό», Αθήνα. Ελληνική Νεφρολογία 2012; 24 (4): 253-260.

**Σκοπός:** Η καρδιαγγειακή νόσος είναι η κύρια αιτία νοσηρότητας και θνησιμότητας των αιμοκαθαρομένων ασθενών. Σκοπός της παρούσας μελέτης ήταν η διερεύνηση της συσχέτισης μεταξύ καρδιαγγειακών νοσημάτων και αρτηριακής σκληρίας σε ασθενείς σε θεραπεία νεφρικής υποκατάστασης της νεφρικής λειτουργίας.

**Ασθενείς - Μέθοδοι:** Μελετήσαμε 96 ασθενείς (34 σε κλασική αιμοκάθαρση, 42 σε αιμοδιαδιήθηση και 20 σε περιτοναϊκή κάθαρση). Η αρτηριακή σκληρία υπολογίσθηκε με την ταχύτητα σφυγμικού κύματος μεταξύ καρωτίδος και μηριαίας αρτηρίας (c-f PWV). Τα επίπεδα της β2-μικροσφαιρίνης (β2Μ) μετρήθηκαν ραδιανοσολογικά και τα επίπεδα της υψηλής ευαισθησίας C-αντιδρώσας πρωτεΐνης (hs-CRP) με ELISA. Η επάρκεια καθάρσεως εκτιμήθηκε με το Kt/V για την ouiria.
Αρτηριακή σκληρία είναι ανεξάρτητος προγνωστικός (B=0,307, OR=1,359, 95.0% CI 1,115-1,657, p=0,002 φρικής υποκατάστασης της νεφρικής λειτουργίας, η ξάρτητοι προγνωστικοί δείκτες παρουσίας ΣΝ μετά δείκτης στεφανιαίας νόσου και καρδιακή ανεπάρκεια (ΚΑ) ή περιφεριακής αγγειοπάθειας (ΠΑ).

Συμπεράσματα: Στους ασθενείς σε θεραπεία νεφρικής υποκατάστασης, η αρτηριακή σκληρία είναι ανεξάρτητος προγνωστικός δείκτης στεφανιαίας νόσου και καρδιακής ανεπάρκειας. Η hs-CRP, συσχετίζεται με τις επίπεδα της β2-μικροσφαιρίνης και υποδηλώνει ότι η φλεγμονή κάθαρση, φλεγμονή.

References


