hsCRP, sICAM-1 and TAFI in Hemodialysis Patients: Linking Inflammation and Hypofibrinolysis to Cardiovascular Events

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Conclusion: This study demonstrates the association of inflammation and hypofibrinolysis with hemodialysis especially in patients with CVD. We found no added therapeutic value for L-arginine at the used dose and duration to ameliorate these cascades of events.

Abstract

Background/Aims: Growing evidence suggests that inflammation, oxidative stress and hypofibrinolysis may have a pivotal role in the high prevalence of cardiovascular disease (CVD) in chronic kidney disease (CKD) patients. This study aims to investigate the association of these processes with the incidence of CVD in hemodialysis (HD) patients and to examine the modulating effect of oral L-arginine in HD patients having CVD. Methods: Blood malondialdehyde (MDA), highly sensitive C-reactive protein (hsCRP), soluble intracellular adhesion molecule-1 (sICAM-1) and thrombin-activatable fibrinolysis inhibitor (TAFI) levels were measured in 12 healthy controls and in 62 CKD patients divided into 15 renal impairment, 21 HD, and 26 HD+CVD. Of the latter, 15 patients received oral L-arginine (15 g/day, 5 g t.i.d.) for 1 month. Results: MDA, hsCRP, sICAM-1 and TAFI were significantly elevated in renal impairment patients. HD and HD+CVD experienced higher levels, but only MDA and TAFI were significantly higher in HD+CVD than HD patients. Only MDA was significantly reduced by 41% after L-arginine intake.

Introduction

Cardiovascular disease (CVD) is the major cause of morbidity and mortality in chronic kidney disease (CKD) patients [1]. The annual mortality rate due to CVD in hemodialysis (HD) and peritoneal dialysis patients is about 9%, which is 10–20 times higher than that observed in the general population [2]. It has been postulated that ‘non-traditional’ risk factors such as oxidative stress, inflammation, endothelial dysfunction and hypofibrinolysis contribute to the high prevalence of CVD in these patients [3].

Over the last several years, the idea that inflammation plays a key role in atherosclerosis has received considerable attention [4]. Now, end-stage renal disease (ESRD) is considered a prototypical situation of chronic inflammatory state [5]. It has been recognized that about 30–50% of predialysis and HD patients have serological evidence...
of an activated inflammatory response, which can be related to renal failure per se or may be a consequence of the dialysis process [6].

C-reactive protein (CRP), a marker of systemic inflammation and a surrogate of underlying cytokine stimulus, has been reported to be associated with all-cause and cardiovascular diseases (CVD) in renal failure [3]. There is accumulating evidence that CRP may be more than just a precise index of inflammation and may contribute directly to endothelial injury and pathogenesis of atherosclerosis [7].

Soluble intracellular adhesion molecule-1 (sICAM-1), a member of Ig-like supergene family of adhesion molecules that are normally expressed by endothelial cells, promotes the adhesion of leukocytes to the inflamed endothelium, which is an early event in the atherosclerosis process [3]. Increased levels of soluble adhesion molecules have been reported in CKD patients, both on conservative therapy and on HD [8]. Moreover, elevated levels of sICAM-1 were observed in patients with history of myocardial infarction and angina pectoris [9].

It has been reported that endothelial function is disturbed in uremic patients with the consequence of development of prothrombotic and hypofibrinolytic state [10]. Hypofibrinolysis in this population is evidenced by increased levels of tissue factor and plasminogen activator inhibitor-1 as well as decreased activity of protein C. However, there are little data in ESRD and HD about the thrombin-activatable fibrinolysis inhibitor (TAFI), a 60-kDa single chain glycoprotein, referred to plasma procarboxypeptidase B [11]. The active form (TAFI a) cleavages C-terminal lysine residues in fibrin necessary for plasminogen binding, and thereby retards plasminogen conversion to plasmin and delays fibrinolysis [12]. As suppression of fibrinolysis is associated with CVD [13], an elevated level of TAFI is a risk factor for venous thrombosis [14]. Evidence supports that TAFI might be an additional member in the pathogenesis of atherosclerosis in CKD.

Moreover, several studies have demonstrated that uremia is associated with enhanced oxidative stress, which has a potential role in the pathogenesis of atherosclerosis [15], mainly through scavenging nitric oxide (NO) and subsequent endothelial dysfunction [16]. Intake of high doses of L-arginine, the NO precursor, has demonstrated beneficial effects in several models of CKD [17].

In a recent article from our laboratory [18], we provided evidence that oxidative stress and asymmetric dimethylarginine are associated with cardiovascular complications in HD patients and that administration of oral L-arginine (15 g/day) significantly improved the cardiovascular markers profile of those patients. In continuation, this study aims to unravel other pathways that play a role in the development of CVD in HD patients, including inflammation and hypofibrinolysis, and to test the therapeutic value of L-arginine in reducing the progression of these cascades.

Methods

Subjects

Sixty-two patients aged 45–69 years with CKD from the Department of Nephrology at Maadi and Kobri El-Obba Military Hospitals, Cairo, Egypt, were included in this study. They comprised 15 nondialysis patients on conservative therapy (renal impairment group), 21 HD patients without CVD (HD group) and 26 HD patients with CVD (HD+CVD group). CV events were defined as acute myocardial infarction, diagnosed by typical clinical and ECG changes, angina pectoris based on typical clinical characteristics or transitory ischemic events, verified by echocardiography. All patients were clinically stable and not under emergency care during the course of the study. Fifteen patients of the HD+CVD group were treated with oral L-arginine (15 g/day, 5 g t.i.d.) [19]. All HD patients were on conventional 4-hour HD sessions, three times weekly with bicarbonate dialysate and heparin as anticoagulant. The control group consisted of 12 male age-matched healthy volunteers. Subjects with diabetes mellitus, chronic liver disease or any serious comitant disease were excluded from the study. The study protocol was approved by the local university ethical committee and informed consent was obtained from all subjects.

Laboratory Methods

Blood samples from controls and patients were collected under fasting conditions. Samples from HD patients were collected from the arteriovenous fistula before the beginning of the first dialysis session of the week. Samples within 30 min were divided into two portions; one portion was collected on Na2-EDTA for TAFI assay, after separation of plasma by centrifugation for 15 min at 2,500 g, and the other portion was collected on plain vacutainer tubes for the assay of the rest of parameters, after separation of sera by centrifugation at 4,000 rpm for 15 min. Plasma and sera were aliquoted and stored at −70°C until assayed.

Assays

Before storage of sera, levels of creatinine, urea, total cholesterol (TC) and triglycerides (TG) were determined using commercially available kits. HDL cholesterol (HDL-C) was determined after precipitation of apolipoprotein B-containing lipoproteins. LDL cholesterol (LDL-C) was calculated by Friedewald equation [20].

Malondialdehyde (MDA) levels, marker of lipid peroxidation, were determined as thiobarbituric acid reactive substances (TBARS) according to the procedure of Uchiyama and Mihara [21]. Serum hsCRP and sICAM-1 were determined by ELISA technique using ACTIVE® kit, supplied by Diagnostic System and Laboratories Inc., Webster, Tex., USA, and Quantikine® kit,
supplied by Research and Diagnostic Systems Inc., Minneapolis, Minn., USA. Plasma TAFI levels were assayed using the ZYMUT-EST® TAFI Ag kit, supplied by Hyphen BioMed, Andresy, France. All ELISA procedures were done by Hyprep® automated ELISA systems, Hyperion Inc., Miami, Fla., USA. The concentrations of hsCRP, sICAM-1 and TAFI were calculated by references to standard curves performed with corresponding recombinant molecule.

Statistical Analysis
All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 9 software. Data are expressed as mean ± SEM. To compare the differences between groups, one-way analysis of variance (ANOVA), followed by LSD post-hoc analysis were used for parametric analysis. The effect of L-arginine administration was evaluated by the paired-sample t test. Correlations were tested by Pearson coefficient. A two-tailed p < 0.05 was considered statistically significant.

Table 1. Clinical and serum laboratory characteristics of the four studied groups; healthy controls, renal impairment group, HD group free from CVD (HD), and HD group suffering from CVD (HD+CVD)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Control</th>
<th>Renal impairment</th>
<th>HD</th>
<th>HD+CVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>12</td>
<td>15</td>
<td>21</td>
<td>26</td>
</tr>
<tr>
<td>Age, years</td>
<td>52.5 ± 0.8</td>
<td>55.53 ± 1.65</td>
<td>54.76 ± 1.44</td>
<td>57.62 ± 0.86</td>
</tr>
<tr>
<td>Duration of dialysis, years</td>
<td>none</td>
<td>none</td>
<td>4.29 ± 0.4</td>
<td>8.3 ± 0.6</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.81 ± 0.03</td>
<td>3.01 ± 0.2a</td>
<td>10.36 ± 0.39b, b</td>
<td>9.83 ± 0.34b, b</td>
</tr>
<tr>
<td>Urea, mg/dl</td>
<td>30.79 ± 1.16</td>
<td>84.18 ± 6.6a</td>
<td>126.28 ± 5.92a, b</td>
<td>136.64 ± 6.59a, b</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>95.46 ± 6.12</td>
<td>113.34 ± 8.75a</td>
<td>133.31 ± 8.07a</td>
<td>167.01 ± 7.76a, b</td>
</tr>
<tr>
<td>TC, mg/dl</td>
<td>142.68 ± 3.78</td>
<td>156.16 ± 6.95a</td>
<td>151.31 ± 6.18</td>
<td>171.36 ± 5.34a, c</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>45.58 ± 0.99</td>
<td>38.51 ± 1.88a</td>
<td>34.71 ± 1.36b</td>
<td>30.35 ± 0.94a, c</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>78 ± 3.72</td>
<td>94.98 ± 5.6</td>
<td>89.93 ± 5.77</td>
<td>107.6 ± 4.62b, c</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
a–c Significant difference from healthy controls, renal impairment group, and HD group, respectively, at p < 0.05.

Table 2. MDA, hsCRP, sICAM-1 and TAFI levels in control subjects, renal impairment, hemodialysis patients free from CVD (HD) and hemodialysis patients suffering from CVD (HD+CVD)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Renal impairment</th>
<th>HD</th>
<th>HD+CVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA, nmol/ml</td>
<td>2.48 ± 0.26</td>
<td>4.41 ± 0.34a</td>
<td>5.77 ± 0.34b, b</td>
<td>6.77 ± 0.47a, c</td>
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<tr>
<td>hsCRP, mg/l</td>
<td>3.01 ± 0.18</td>
<td>11.96 ± 1.5a</td>
<td>14.04 ± 1.63a, b</td>
<td>16.25 ± 2.11a</td>
</tr>
<tr>
<td>sICAM-1, ng/ml</td>
<td>128.44 ± 3.63</td>
<td>223.55 ± 13.76a,a</td>
<td>336.15 ± 16.66a,b</td>
<td>356.07 ± 18.66a,b</td>
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<tr>
<td>TAFI Ag, %</td>
<td>60.25 ± 3.27</td>
<td>85.89 ± 4.9a</td>
<td>99.96 ± 2.94a,b</td>
<td>146.35 ± 3.99a,b,c</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
a–c Significant difference from healthy controls, renal impairment group, and HD group, respectively, at p < 0.05.

Results

Baseline Parameters
Baseline characteristics of control, renal impairment, HD and HD+CVD subjects are shown in table 1. Serum creatinine and urea levels were significantly increased in the renal impairment group and dramatically elevated in HD patients with or without CVD when compared with healthy controls. There was no significant difference in serum urea and creatinine between HD and HD+CVD groups. Regarding the lipid profile, TG levels were significantly increased in HD and HD+CVD groups to about 1.4- and 1.7-fold of the control level, respectively. HDL-C was significantly decreased in all the studied groups when compared with controls. Concerning the levels of TC and LDL-C, neither renal impairment nor...
HD patients showed significant differences from controls. Only HD+CVD group showed significant elevation in TC and LDL-C levels.

**Biomarkers of Inflammation and Fibrinolysis**

As shown in Table 2, all CKD patients showed significantly elevated levels of MDA as compared with normal controls (2.48 ± 0.26 nmol/ml; p < 0.01). The MDA levels in renal impairment, HD, and HD+CVD were 4.41 ± 0.34, 5.77 ± 0.34 and 6.77 ± 0.47 nmol/ml, respectively. Regarding hsCRP, their levels were significantly increased in all CKD patients. The elevation reached 3.97-, 4.66- and 5.28-fold of the control level for renal impairment, HD, and HD+CVD groups, respectively (p < 0.05). However, there was no significant difference in hsCRP levels among CKD groups.

Concerning serum sICAM-1 levels, all CKD patients showed significant increase reaching to 174, 262 and 277% of the control level for renal impairment, HD, and HD+CVD patients, respectively (p < 0.005). The effect of HD was well demonstrated by the significant increase in levels of sICAM-1 to 150–160% of the renal impairment group for HD and HD+CVD patients. The same pattern was observed for TAFI, but with lower increases, and the difference in the HD+CVD group was highly significant compared to the HD group.

**Effect of L-Arginine Administration**

After 1 month of L-arginine treatment in a group of HD+CVD patients, a significant reduction in urea and TG levels was observed (Table 3). The most notable change in the studied parameters was observed in the MDA levels, reaching 41% of the average pretreatment level.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before L-arginine</th>
<th>After L-arginine</th>
</tr>
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<tbody>
<tr>
<td>Creatinine, mg/dl</td>
<td>9.9 ± 0.47</td>
<td>9.59 ± 0.47</td>
</tr>
<tr>
<td>Urea, mg/dl</td>
<td>142.93 ± 8.5</td>
<td>118.64 ± 4.73*</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>168.78 ± 7.33</td>
<td>161.05 ± 7.28</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>166.7 ± 9.61</td>
<td>149.06 ± 10.2*</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>30.59 ± 1.42</td>
<td>31.4 ± 1.22</td>
</tr>
<tr>
<td>MDA, mmol/ml</td>
<td>6.3 ± 0.58</td>
<td>3.71 ± 0.51*</td>
</tr>
<tr>
<td>hsCRP, mg/l</td>
<td>15.9 ± 2.8</td>
<td>15.51 ± 2.72</td>
</tr>
<tr>
<td>sICAM-1, ng/ml</td>
<td>351.73 ± 22.38</td>
<td>349.78 ± 19.37</td>
</tr>
<tr>
<td>TAFI Ag, %</td>
<td>146.51 ± 5.38</td>
<td>148.45 ± 6.1</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. * Statistically significant from before oral L-arginine administration at p < 0.05.
Evidence from experimental and clinical studies has been accumulating that CRP is not just a sensitive marker of systemic inflammation but also an amplifier of it [26]. Any inflammatory stimuli would prompt the release of IL-1, IL-6, and TNF, which can stimulate the liver to produce CRP [24]. Many cardiologists believe now that serum CRP level is the strongest univariate predictor of the risk of cardiovascular events [26]. CRP may participate directly in the pathogenesis of atherosclerosis through a variety of mechanisms [7]. From these, CRP activates complement system, binds to degraded LDL particles and colocalize with lipid particles in early atherosclerotic lesions [27]. CRP also induces expression of adhesion molecules [28] that promote firm adhesion of leukocytes to the inflamed endothelium, one key step in the initiation of atherosclerosis. The latter can explain the linear relationship between hsCRP and sICAM-1 demonstrated in the correlation study (fig. 1b). The failure to demonstrate a significant difference in hsCRP and sICAM-1 between HD and HD+CVD could be related to the small sample size and to the fact that all HD+CVD patients were under treatment with nitro-compounds; these in the form of transdermal nitroglycerin have demonstrated anti-inflammatory action in patients with peripheral vascular disease and was able to reduce the plasma levels of CRP [29].

The results of the study also demonstrate a positive association between MDA and sICAM-1. A reasonable explanation can be inferred from the NO-quenching effect of free radicals generated during oxidative stress. NO has an inhibitory action on adhesion molecules expression [30]. Previous studies have shown that elevated levels of sICAM-1 are associated with CV events in nonrenal individuals [31] as well as mortality rates in pre-dialysis ESRD patients with CVD [32] and in chronic hemodialysis patients [33].

This study also revealed an increase in TG levels in the HD and HD+CVD groups as well as a significant reduction in HDL-C levels in all CKD patients. These abnormalities are mainly due to decreased lipolytic enzyme activities as a consequence of elevated proinflammatory cytokines. Both systemic inflammation and low HDL-C levels have been regarded as important CV risk factors [34]. The study demonstrated a negative correlation between serum HDL-C and both hsCRP and sICAM-1 (data not shown). These observations could be explained by many theories. First, HDL-C is known to have anti-inflammatory action and a direct inhibitory effect on adhesion molecules expression [35]. Second, inflammatory condition is characterized by increased levels of serum
amyloid A which replaces apo A-1, the major protein of HDL, producing a chemoattractive form of HDL [36]. Hence, reduced levels of HDL-C together with its structural abnormalities are associated with enhanced inflammatory response and increased levels of adhesion molecules, including sICAM-1. Based on the above results, an association between inflammation, adhesion molecules, dyslipidemia and atherosclerosis could be assumed.

One important goal of this study is to investigate the levels of plasma TAFI antigen in CKD patients as a recent marker of hypofibrinolysis. Results revealed that all CKD patients in the study have elevated levels of TAFI Ag as compared to controls. The higher levels of TAFI in the HD+CVD group in comparison with the HD and renal impairment groups may identify its involvement in CV events associated with this population. Elevated levels of TAFI have been reported in patients with angina pectoris and angiographically confirmed coronary artery disease [37]. In addition, previous studies considered TAFI as a risk factor for deep vein thrombosis [14]. The mechanism of increased TAFI and its exact role in the pathogenesis of CV events in CKD patients is still not fully clear. However, this study demonstrated a positive correlation of TAFI with TG, MDA and sICAM-1 and a negative one with HDL-C. Beside hepatic cells, it was also reported that endothelial cells can secrete TAFI in response to oxidative stress, dyslipidemia and other metabolic abnormalities in uremia; and this could explain in part the previous associations and the increase of TAFI in patients with chronic renal failure [38]. Elevated circulating TAFI Ag and activity has also been reported in nephritic syndrome [39].

In a previous work [18], L-arginine supplementation to HD patients with CVD provided significant success in reducing the markers of oxidative stress and endothelial dysfunction (asymmetric dimethyl arginine, homocysteine, myeloperoxidase, and MDA) in the blood of these patients after 1 month of treatment. By comparison and under the same conditions of the aforementioned study, L-arginine supplementation in the present study did not demonstrate significant improvement in the inflammation and fibrinolysis associated with HD. It did not aggravate them either. The failure to demonstrate significant effects of L-arginine on levels of hsCRP and sICAM-1 agrees with two previous studies in healthy women [40] and in patients with coronary artery disease [41]. The short duration and insufficient dose of L-arginine might represent an explanation. One exception from these negative effects is the highly significant reduction in MDA concentrations by L-arginine, which was also demonstrated in our previous study [18]. These results are consistent with in vitro studies that showed that L-arginine supplementation prevents preferential superoxide production in endothelial cells exposed to near physiological levels of oxidized-LDL [42].

In conclusion, the most notable findings of this study are:

1. Markers of inflammation (hsCRP, sICAM-1) and hypofibrinolysis (TAFI) are elevated in CKD patients.
2. These markers are positively correlated with each other and with oxidative stress marker (MDA).
3. Inflammation and hypofibrinolysis persist under dialysis treatment.
4. HD patients with established CVD complications have higher levels of the risk parameters (TAFI and MDA) and tendency for elevation for other parameters, which might need larger sample size for significance.
5. Oral L-arginine administration, at the provided dose and duration, has no added therapeutic value on the studied inflammation and hypofibrinolysis cascades. Its improving effect on oxidative stress and endothelial dysfunction was provided in the earlier report [18].

Our main recommendations are to consider chronic inflammation and hypofibrinolysis in the management regimen of HD patients and to look for a comprehensive approach to minimize the CV complications associated with HD treatment.

References