Plasma 8-OHdG Levels Correlate With Endothelial Dysfunction and Atherosclerosis in Peritoneal Dialysis Patients

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Abstract

Background: Accelerated atherosclerosis is a major cause of increased cardiovascular morbidity and mortality in patients on chronic peritoneal dialysis (PD). This study aims to investigate the relationship among oxidative stress, endothelial dysfunction and atherosclerosis in PD patients.

Methods: Eighty non-diabetic PD patients and twelve healthy controls were enrolled in the trial. Plasma 8-OHdG and ET-1 levels were quantified using enzyme-linked immunosorbent assay and radioimmunoassay respectively. The eNOS activities were measured by nitrate reductase assay. Carotid intimal-medial thickness (CIMT) was assessed by quantitative carotid artery ultrasonography.

Results: The mean age of the PD patients was 41.6±14.03 years and that of controls 40.92±5.32 years respectively (P >0.05). Plasma levels of 8-OHdG and ET-1 increased in patients on maintenance peritoneal dialysis. The mean plasma 8-OHdG concentrations were 39.33±9.34 ng/ml in PD patients and 7.25±0.97 ng/ml in the control group while the mean plasma ET-1 were 139.35±31.12 pg/ml in PD patients and 16.00±1.41 pg/ml in the control group respectively, (P <0.01). The eNOS activities were significantly lower in PD patients compared to controls (12.57±1.76 u/ml vs 14.25±1.86 u/ml, P <0.01). CIMT was higher in PD patients as compared to controls (1.21±0.40mm vs 0.92±0.29mm, P <0.01). Linear regression analysis showed that the levels of plasma 8-OHdG had a significant positive correlation with plasma ET-1 and CIMT, but a negative correlation with plasma eNOS or Kt/V.

Conclusion: The endothelial dysfunction in PD patients was significant and it was positive correlated with the oxidative stress indicted by the plasma levels of 8-OHdG. The levels of plasma 8-OHdG could serve as an independently risk factor for endothelial dysfunction and atherosclerosis in PD patients.

Keywords: Peritoneal Dialysis; 8-OHdg; Endothelial Dysfunction; Carotid Intimal Medial Thickness; Atherosclerosis.

Introduction

Accelerated atherosclerosis is a leading cause of increased cardiovascular morbidity and mortality in peritoneal dialysis (PD) patients. Traditional factors such as hypertension, hyperlipidemia, diabetes, smoking, and advanced age are thought to contribute to accelerated atherosclerosis. However, the exact mechanisms responsible for this accelerated development of atherosclerosis have remained elusive. Novel risk factors, such as excess production of reactive oxygen species (ROS), endothelial dysfunction and inflammation have been increasingly associated with this process [1,2].

Increased oxidative stress (OS) and endothelial dysfunction have been recently suggested to be key factors in the pathogenesis and development of atherosclerosis in end-stage renal disease (ESRD) patients [3]. Oxidative stress is the result of an imbalance between generation and removing of reactive oxygen species. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Endothelial dysfunction is an early initiated event in atherosclerosis as well as a risk factor for future cardiovascular disease. The presence of increased OS and endothelial dysfunction in ESRD patients has been demonstrated by many studies and oxidative stress, by causing NO breakdown, can be a major mechanism of endothelial dysfunction. However, very few studies have been performed to investigate the role of these important non-traditional factors in accelerated atherosclerosis among PD patients.

To delineate the effects of oxidative stress and endothelial dysfunction on the atherosclerosis in PD patients, we assessed the levels of plasma 8-OHdG, ET-1, and eNOS activities as well as carotid intima-media thickness (CIMT) to determine the association of oxidative stress, endothelial dysfunction and accelerated atherosclerosis. Our findings not only clearly identify oxidative stress and endothelial dysfunction as the key players in the development of accelerated atherosclerosis but also suggest antioxidant therapy may reduce the incidence of cardiovascular disease in PD patients.

Materials and Methods

Subjects

This was a cross–sectional, cohort, comparison study and conducted at the Second Hospital of Hebei Medical University in Shijiazhuang, China. The study was approved by the Ethics Committee on Human Research at Second Hospital of Hebei Medical University. All study subjects signed a written, informed consent prior to enrollment. Eighty PD patients with ESRD and being on PD for more than 6 months (aged 41.6±14.03 years; 24 men and 56 women) as well as 12 age- and sex-matched healthy controls free from any signs of chronic disease after clinical examination and laboratory checkup (aged 40.92±5.32; 5 men and 7 women) participated in the study. All PD patients were performing 4 h 2 L exchanges per day dialysis with standard acidic, lactate-based glucose dialysis solution. They were on the Baxter Twin Bag system. Dwell times were generally 4-6h during the day. All study subjects were non-smokers and did not consume alcohol regularly. None of the subjects received antibiotics, corticosteroids, anti-inflammatory drugs, cytotoxic drugs and vitamins during the study. Kt/V was adopted to evaluate the dialysis adequacy.
Exclusion criteria: patients with any systemic illness such as diabetes, malignancy, lupus, vasculitis were excluded. Patients with intravenous iron treatment or with any infection disease in the past month were also excluded.

Laboratory Examinations

Measurement of plasma 8-hydroxydeoxyguanosine (8-OHdG) levels

8-OHdG levels were measured by using a competitive in vitro enzyme-linked immunosorbent assay (ELISA) kit (Bioxytech, OXIS Health Products, Inc., Portland, OR, USA) according to the manufacture’s protocol. In brief, 50μl plasma sample and 50μl of reconstituted primary antibody were added to each well of 8-OHdG-coated microtitre plates and incubated at 37°C for 1 h. Plates were washed three times with phosphate-buffered saline (PBS) followed by adding horseradish peroxidase-conjugated secondary antibody. After incubation at 37°C for another hour, unbound secondary antibody was removed, and the plate was washed again three times. The amount of antibody bound to the plate was determined by the development of color intensity after the addition of substrate containing 3,3',5, '-tetramethyl-benzidine using a computer-controlled spectrophotometric plate reader at the wavelength of 450 nm. The concentration of 8-OHdG was interpolated from a standard curve. The detection range of the ELISA assay was 0.5–200ng/ml.

Measurement of plasma Endothelin-1 levels

Plasma ET-1 concentrations were determined by use of a radioimmunoassay kit (Beijing North Institute of Biotechnology, Beijing, China) according to its manufacture’s protocol. In short, 100μl of plasma sample were added into duplicate tubes followed by adding primary antibody. After incubation for 16-24 hours at 4°C, 100μl of 125I-peptide tracer solution were added followed by incubation for 16-24 hours at 4°C. Then the tubes were incubated for 90 minutes at 4°C. All the supernatants were carefully removed immediately following centrifugation. Counts Per Minute (Cpm) of all the pellets were counted by using a γ-counter. Plasma ET-1 concentrations were calculated from the stand curve. The detection range of the RIA assay was 0.5–12080pg/ml.

Measurement of plasma endothelial nitric oxide synthase activity

Plasma eNOS activities were measured using colorimetric nitrate reductase assay by Nanjing Jiancheng Biochemical Co (Nanjing, China).

Measurement of carotid artery intimal-medial thickness

Quantitative ultrasound measurements for both sides of carotid artery intimal thickness were performed using ATL HDI 5000 SonoCT/XRES Color Doppler System (Bothell, WA) by a single expert sonographer. The sonographer was blinded to the subject’s identity.

Statistical analysis

Age, sex, medications, blood pressure, hemoglobin (Hb), albumin, cholesterol, Calcium (Ca), phosphate (Phos), blood urea nitrogen (BUN) and serum creatinine (Scr) were recorded. Clinical characteristics were reported as means ± standard deviations for continuous variables and frequencies for categorical variables. Statistical analysis was performed on SPSS (IBM, Armonk, NY) version 13.0. Differences between the groups were examined by one-way ANOVA or unpaired Student's t-test when necessary and chi-square tests for continuous and categorical variables, respectively. A multiple linear regression analysis was used to determine the independent predictor of CIMT. Results with p<0.05 were considered statistically significant in all analyses.

Results

Clinical characteristics of patients enrolled in the study

Clinical and demographic characteristics of the PD patients and control groups are given in Table 1. The mean dialysis duration was 24 months (range 13–44 months) in PD group. The causes of ESRD were primary glomerulonephritis (60 cases), polycystic kidney (3 cases), chronic interstitial nephritis (1 case), lupus nephritis (1 case), unknown causes (15 cases).

Carotid intimal-medial thickness

Results of CIMT are given in Tables 1 & 2. The carotid intimal-medial thickness was significantly greater in PD patients than in control subjects. PD patients had significant higher morbidity of atherosclerosis than control patients. CIMT was 1.21±0.40mm in PD group and 0.92±0.29mm in the control group, P<0.01. Sixty-one (76.25%) PD patients were diagnosed with carotid atherosclerosis while only three (25.00%) in the control group, P<0.01.

Plasma levels of 8-OHdG, ET-1 and eNOS activities

Results of plasma 8-OHdG, ET-1 and eNOS are shown in tables 3 and 4. The levels of plasma 8-OHdG and ET were statistically higher in PD...
Table 1. Clinical characteristics of patients enrolled in the study

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=12)</th>
<th>PD group (n=80)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>5/7</td>
<td>24/56</td>
<td>0.633</td>
</tr>
<tr>
<td>Age(years)</td>
<td>40.92±5.32</td>
<td>41.6±14.03</td>
<td>0.757</td>
</tr>
<tr>
<td>SBP(mmHg)</td>
<td>132.50±6.74</td>
<td>147.24±13.28</td>
<td>0.000*</td>
</tr>
<tr>
<td>DBP(mmHg)</td>
<td>82.33±4.50</td>
<td>88.5±10.9</td>
<td>0.001*</td>
</tr>
<tr>
<td>Hb(g/L)</td>
<td>132.50±11.38</td>
<td>111.18±12.34</td>
<td>0.000*</td>
</tr>
<tr>
<td>ALB(g/L)</td>
<td>49.67±2.39</td>
<td>36.41±5.06</td>
<td>0.000*</td>
</tr>
<tr>
<td>CHOL(mmol/L)</td>
<td>5.17±0.83</td>
<td>5.18±1.6</td>
<td>0.967</td>
</tr>
<tr>
<td>BUN(mmol/L)</td>
<td>5.67±0.89</td>
<td>22.26±6.30</td>
<td>0.000*</td>
</tr>
<tr>
<td>Scr(umol/L)</td>
<td>69.83±16.62</td>
<td>962.98±329.13</td>
<td>0.000*</td>
</tr>
<tr>
<td>Ca(mmol/L)</td>
<td>2.0±0.01</td>
<td>2.05±0.34</td>
<td>0.21</td>
</tr>
<tr>
<td>Phos(mmol/L)</td>
<td>1.0±0.01</td>
<td>1.59±0.32</td>
<td>0.000*</td>
</tr>
<tr>
<td>hsCRP(mg/L)</td>
<td>2.50±0.90</td>
<td>4.85±1.63</td>
<td>0.000*</td>
</tr>
<tr>
<td>CIMT(mm)</td>
<td>0.9±0.29</td>
<td>1.21±0.40</td>
<td>0.006*</td>
</tr>
<tr>
<td>Total KT/V</td>
<td>—</td>
<td>1.92±0.37</td>
<td>—</td>
</tr>
<tr>
<td>Time (months)</td>
<td>—</td>
<td>24(13–44)</td>
<td>—</td>
</tr>
</tbody>
</table>

*P<0.01

Table 2. Atherosclerosis in carotid artery in PD and control patients

<table>
<thead>
<tr>
<th></th>
<th>CIMT&lt;1.0 mm(case)</th>
<th>CIMT≥1.0 mm (case)</th>
<th>Total (case)</th>
<th>Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD group</td>
<td>19</td>
<td>61</td>
<td>80</td>
<td>76.25*</td>
</tr>
<tr>
<td>Control group</td>
<td>9</td>
<td>3</td>
<td>12</td>
<td>25.00</td>
</tr>
</tbody>
</table>

Table 1. Clinical characteristics of patients enrolled in the study

Note: Time: On dialysis

Table 2. Atherosclerosis in carotid artery in PD and control patients

Note: * P<0.01

patients than that in their counterparts (39.33±9.34ng/ml vs 7.25 ±0.97ng/ml, 139.35±31.12pg/ml vs 16.00±1.41pg/ml, respectively P < 0.01, Fig 1). Further analysis showed that the levels of plasma 8-OHdG and ET-1 in PD patients with carotid atherosclerosis dramatically increased compared to those without carotid atherosclerosis (42.83±7.70ng/ml vs 28.07±3.15ng/ml and 147.97±28.49pg/ml vs 111.69±22.05pg/ml respectively, P<0.01). On the other hand, plasma eNOS activities were significantly decreased in PD patients than in control group (12.57±1.76u/ml vs 14.25±1.86u/ml, P < 0.01, Fig 1). Similarly, PD patients with atherosclerosis showed statistically lower eNOS activities than those without (12.14±1.52u/ml vs 13.96±1.80u/ml, P<0.01).
Correlation analysis

Linear regression analysis was performed to identify the correlations between characteristics of patients, CIMT and the levels of plasma 8-OHdG, ET-1 and eNOS activities. The levels of plasma 8-OHdG in PD patients were correlated positively with ET-1 as well as CIMT, and the correlation was statistically significant (P<0.01, Fig 2). In addition, the levels of plasma 8-OHdG had significant positive correlations with age (r=0.697, P=0.000), dialysis duration (r=0.752, P=0.000), hsCRP (r=0.532, P=0.000), systolic blood pressure (r=0.350, P=0.001), diastolic blood pressure (r=0.269, P=0.000), while negative correlation with eNOS (r=-0.723, P=0.000), KT/V (r=-0.367, P=0.001), but without any correlation with ALB, Hb, Scr, BUN, CHOL, Ca, or Phos (P>0.05).

The levels of plasma ET-1 in PD patients were positively correlated with age (r=0.639, P=0.000), dialysis duration (r=0.569, P=0.000), hsCRP (r=0.438, P=0.000), systolic blood pressure (r=0.364, P=0.001), diastolic blood pressure (r=0.280, P=0.002), CIMT (r=0.585, P=0.000), 8-OHdG (r=0.753, P=0.000), but negatively correlated with KT/V (r=-0.285, P=0.010), eNOS activity (r=-0.646, P=0.000). There was no correlation between plasma ET-1 levels and ALB, Hb, Scr, BUN, CHOL, Ca, or Phos (P>0.05).

The levels of plasma eNOS in PD patients were negatively correlated with age (r=-0.656, P=0.000), dialysis duration (r=-0.599, P=0.000), systolic blood pressure (r=-0.299, P=0.007), diastolic blood pressure (r=-0.209, P=0.043), hsCRP (r=-0.408, P=0.000), 8-OHdG (r=-0.723, P=0.000), CIMT (r=-0.579, P=0.000); positively correlated with KT/V (r=0.329, P=0.003), but not correlated with ALB, Hb, Scr, BUN, CHOL, Ca, or Phos (P>0.05).

Discussion

Chronic renal disease is associated with accelerated cardiovascular risk and the cardiovascular diseases are the most common cause of morbidity and mortality in PD patients [5]. It is well known that accelerated atherosclerosis (AS) is one of the major factors associated with morbidity and mortality of cardiovascular disease. Studies have shown that thickened carotid intima-media thickness is an early independent predictor in ESRD patients and may be used to...
predict the risk of cardiovascular events [6]. We found that the morbidity of carotid artery atherosclerosis is as high as 76.25% in ESRD patients receiving maintenance peritoneal dialysis, suggesting that patients receiving peritoneal dialysis may have a higher risk of cardiovascular diseases. This finding is supported by Guo’s report [7] which showed that 69.09% patients receiving maintenance hemodialysis had carotid plaques.

Increased oxidative stress has been shown in patients treated with continuous ambulatory peritoneal dialysis (CAPD) by Boudouris et al [8]. Oxidative stress is now considered as one of the most important pathogenetic mediator of atherosclerosis among non-traditional cardiovascular risk factors. In addition, oxidative stress also contributes to the ultrafiltration failure [8,9] of peritoneal membrane. However, factors caused the increase of oxidative stress in PD patients are unclear. 8-OHdG is the special metabolic end product of DNA oxidative damage and is universally acknowledged as a new type of sensitive biomarkers for evaluation oxidative stress in the body. Our research shows that plasma 8-OHdG levels increased significantly in peritoneal dialysis patients compared to those in the control group, which were about as 5.4 times high as those in the control group. The plasma 8-OHdG levels were also positively correlated with age, systolic pressure, diastolic pressure and hs-CRP. Previous study [7] demonstrated that 8-OHdG levels increased in the ESRD patients receiving maintenance hemodialysis and our data suggest this phenomenon also exists in peritoneal dialysis patients. Moreover, oxidative stress is closely associated with the inflammation of patients with peritoneal dialysis and high blood pressure. Oxidative stress has also been shown being an independent risk factor of high systolic and diastolic blood pressure in patients with peritoneal dialysis [10,11]. It is very likely that the inflammatory process induces oxidative stress and oxidative stress enhances inflammatory reaction. It has been suggested that both the inflammation and oxidative stress involved in the pathogenesis of high blood pressure while hypertension itself or factors associated with hypertension such as fluid retention aggravate the oxidative stress in PD patients. The synergetic effects of oxidative stress and hypertension further lead to the failure of peritoneal membrane.

It has been well documented that oxidative stress and vascular endothelial dysfunction are common features of patients with chronic kidney disease (CKD) [12,13]. This study demonstrated that PD patients still exhibit endothelial dysfunction given that the plasma levels of ET-1 increased significantly while eNOS activities reduced dramatically in these patients. This result suggests dialysis itself is not enough to block the progression of chronic kidney diseases and novel therapy against oxidative stress needs to be developed. The exact pathogenesis of endothelial dysfunction in patients with ESRD is unclear but oxidative stress plays the leading role in vascular endothelial cell damage, the initiation and development of endothelial dysfunction in addition to many other factors, such as high blood pressure, high blood sugar, the accumulation of toxin, lipid metabolic disorder, and inflammation [14].

Interestingly, Malik et al [15] has shown that oxidative stress is one of the critical factors in the pathogenesis of atherosclerosis. Further study demonstrated that plasma levels of oxidative stress marker 8-OHdG in hemodialysis patients were positively correlated with CIMT and it is possible to be adopted as one of the subclinical predictors for atherosclerosis [16]. Our results and other report [17] showed that, oxidative stress indicated by 8-OHdG or ADMA levels is the independent risk factor of CIMT in PD patients. It has been reported that peritoneal dialysis patients exhibit vascular endothelial dysfunction even those there was no evidence of atherosclerosis disease [3]. Our study also demonstrated that CIMT positively correlated with 8-OHdG in PD patients, and the plasma 8-OHdG levels were significant higher in PD patients with arterial stiffness than those without. The plasma levels of 8-OHdG statistically associated with ET-1 and eNOS activities in PD patients, which indicate that oxidative stress and endothelial dysfunction play a critical role in the development of cardiovascular disease in PD patients. Our results that the endothelial dysfunction in PD patients positively correlated with CIMT support the idea that the endothelial dysfunction is an early sign of atherosclerosis.

There are a number of limitations to our study. The small sample size may limit the applicability of the findings to larger population-level cohorts. Given the cross-sectional study, the progress of oxidative stress has not been observed during peritoneal dialysis. Therefore, potential non-sustained events may have been counted, altering the power of the oxidative stress marker for prediction atherosclerosis.

In summary, PD patients have significant higher oxidative stress, greater endothelial dysfunction, and increased atherosclerosis. These abnormalities are closely associated with each other and enhance the process of atherosclerosis development. Oxidative stress participates in the occurrence and development of the vascular endothelial dysfunction, leading to the initiation of atherosclerosis in PD patients. Plasma 8-OHdG, an oxidative stress marker, is an independently risk factors for atherosclerosis in PD patients. Further study should be conducted to evaluate antioxidant therapy in ESRD patients receiving CAPD to test if antioxidant drugs protect the vascular endothelial function, reduce the incidence of cardiovascular diseases, and improve the survival rate in PD patients.

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References


