Plasma Pentosidine Is Associated with Inflammation and Malnutrition in End-Stage Renal Disease Patients Starting on Dialysis Therapy

MOHAMMED E. SULIMAN, OLOF HEIMBÜRGER, PETER BÁRÁNY, BJÖRN ANDERSTAM, ROBERTO PECOITS-FILHO, ERNESTO RODRÍGUEZ AYALA, A. RASHID QURESHI, INGELA FEHRMAN-EKHOLM, BENGT LINDHOLM, and PETER STENVINKEL
Divisions of Renal Medicine and Baxter Novum, Department of Clinical Science, Karolinska Institutet, Huddinge University Hospital, Stockholm, Sweden.

Abstract. Pentosidine is an advanced glycation end-product (AGE), formed by glycosylation and oxidation, that accumulates markedly in end-stage renal disease (ESRD). It has been speculated that AGE and carbonyl stress contributes to long-term complications such as cardiovascular disease (CVD) in ESRD patients. This study determined plasma levels of pentosidine as well as the presence of inflammation (CRP ≥ 10 mg/L), clinical CVD (CVDclin), and malnutrition (subjective global assessment [SGA] > 1) in a cohort of 191 ESRD patients, median age of 55 yr (range, 23 to 70 yr) and median GFR = 7 ml/min (range, 2 to 17 ml/min), close to start of renal replacement therapy. Fifty-one elderly subjects, median age of 82 yr (range, 71 to 110 yr), with mild renal impairment, median GFR = 67 ml/min (range, 38 to 113 ml/min), were also studied for comparative analysis of plasma pentosidine. The plasma pentosidine content was elevated in all patients compared with the levels in the elderly subjects and were negatively correlated with GFR both in the ESRD patients (Rho = −0.24; P < 0.01; n = 159) and in the elderly subjects (Rho = −0.31; P < 0.05). Moreover, the plasma pentosidine content was correlated with age in the ESRD patients (Rho = 0.26; P < 0.001) and in the elderly subjects (Rho = 0.44; P < 0.001). The 63 malnourished ESRD patients (35%) had a significantly higher (P < 0.05) median plasma pentosidine than the well-nourished patients (39 versus 27 pmol/mg albumin). Similarly, 73 inflamed patients (38%) had a significantly higher (P < 0.001) median pentosidine content compared with 118 non-inflamed patients (37 versus 24 pmol/mg albumin). Also, the plasma pentosidine content showed weak but significant positive correlations with CRP (Rho = 0.28; P < 0.0001), fibrinogen (Rho = 0.23; P < 0.01; n = 126), IL-6 (Rho = 0.22; P < 0.01; n = 169), and soluble vascular cellular adhesion molecule-1 (Rho = 0.38; P < 0.001; n = 74). On the other hand, no significant differences in plasma pentosidine content were noted between the patients with and those without CVDclin (32 versus 27 pmol/mg albumin, respectively). Analyses of all-cause mortality, by Kaplan-Meier, showed that mortality was not linked to the plasma pentosidine content. Moreover, survival analysis by the Cox regression model showed that age (P < 0.001), diabetes mellitus (P < 0.01), malnutrition (P < 0.01), and CVDclin (P < 0.01) independently predicted poor outcome, whereas an elevated plasma pentosidine content did not. The present study shows that an elevated plasma pentosidine content in ESRD patients is significantly associated with both inflammation and malnutrition and confirms that low residual renal function and high age further contribute to an increased plasma pentosidine content. However, in this small cohort, the plasma pentosidine content did not predict outcome. Thus, accumulation of plasma pentosidine is unlikely to be an appropriate clinically useful marker to predict mortality in ESRD patients.

It is now well established that the extremely high cardiovascular risk in end-stage renal disease (ESRD) patients cannot be fully explained by traditional risk factors, such as diabetes mellitus (DM), dyslipidemia, and hypertension. Thus, several putative nontraditional risk factors for accelerated atherosclerosis, such as advanced glycation end products (AGE), oxidative stress, malnutrition, and inflammation, have recently attracted increased interest. Carbohydrate-derived AGE constitute a heterogeneous class of structures, such as pentosidine, Nε-carboxymethyl-lysine (CML), and imidazolone, which are formed by nonenzymatic glycation and oxidation reactions between carbohydrate-derived carbonyl compounds and protein amino groups (Maillard reaction). AGE accumulate in aging, degenerative diseases, and in diabetic patients with sustained hyperglycemia (1). The interest in the pathogenic role of AGE in ESRD increased when elevated content of pentosidine, a major glyoxidation product, was demonstrated in the tissues and plasma of ESRD patients (2). In ESRD patients, the AGE levels are apparently independent of the
serum glucose concentrations (3,4). In uremia, the circulating pentosidine accumulates as both protein-linked and in free form. The kidney is of major importance for excretion of free pentosidine (4); however, the mechanism(s) for removal of protein-linked pentosidine and other protein-linked AGE remain unclear. The cause(s) for accumulation of AGE in ESRD has not been fully delineated (4,5). Nevertheless, it has been speculated that renal insufficiency leads to AGE accumulation due to decreased removal of AGE precursors and/or increased generation of AGE through oxidative stress (6,7).

Although studies in DM indicate that the accumulation of AGE is strongly associated with diabetic complications (3), there is not yet any solid evidence that AGE contribute to long-term complications in ESRD. However, AGE have been implicated in the pathogenesis of dialysis-related amyloidosis (8), and the cardiovascular system may also represent a potential target of AGE. Indeed, immunohistochemical studies by Miyata et al. (9) have shown that pentosidine, as well as other AGE, are present in the neointima of injured carotid artery. On the other hand, in a recent clinical study, Zoccali et al. (10) did not find any relation among plasma pentosidine and intima media thickness and the number of atherosclerotic plaques in hemodialysis (HD) patients. Moreover, Schwedler et al. (11) have recently reported that high levels of CML and total serum fluorescence AGE were not related to a worse survival rate in HD patients.

In the present prospective study, we investigated if the plasma content of pentosidine, a glycoxidation product, analyzed at start of dialysis treatment could be used as a predictor of mortality with a predictive power of the same magnitude as other well-established predictors of outcome in ESRD patients. Moreover, we investigated the relationships between plasma pentosidine content and the presence of inflammation, malnutrition, and cardiovascular disease (CVD), respectively, at start of dialysis treatment. In addition, to better understand the possible effect of the age-related decline in renal function on the plasma pentosidine content, we also studied a separate group of elderly subjects with normal to moderately reduced renal function. These results are presented in comparative analyses.

**Materials and Methods**

**Subjects**

One hundred ninety-one ESRD patients (121 male patients), with a median age 55 yr (range, 23 to 70 yr) and with a median GFR of 7 (2 to 17 ml/min), were studied close to the start of renal replacement therapy (RRT). Patients > 70 yr of age as well as those with liver dysfunction and active infectious disease were excluded. In the present study, post-hoc analyses were done in the patients participating in an ongoing prospective study (12) in whom plasma pentosidine values at baseline were available. Sixty-two patients (32%) had clinical signs of cerebrovascular, cardiovascular, and/or peripheral vascular disease at the start of the study and were grouped as clinical cardiovascular disease (CVDclin). Of the 62 patients, 19 had suffered from cerebrovascular disease (stroke and/or history of transient ischemic attack). Twenty-one had one or more myocardial infarctions as defined by the presence of chest pain, confirmatory electrocardiograms, and enzyme courses. Twenty-six had clinical signs of ischemic heart disease (angina pectoris) and/or had undergone percutaneous coronary angiography and/or had undergone coronary artery bypass graft. Twenty-one had clinical signs of peripheral ischemic atherosclerotic vascular disease, and three patients had a history of an aortic aneurysm. Most patients were on antihypertensive medications as well as other commonly used drugs in terminal chronic renal failure such as phosphate and potassium-binders, diuretics, and vitamin B, C, and D supplementation.

Fifty-one elderly subjects (31 male patients), with normal to moderately reduced renal function (the median GFR was 67 (range 38 to 113) ml/min) and with a median age of 82 yr (range, 71 to 110 yr), served as a group for comparative analyses of the effect of the age related decline of GFR on the plasma pentosidine content. The local Ethics Committee of Karolinska Institutet at Huddinge University Hospital approved the study protocol, and informed consent was obtained from each ESRD patient and elderly subjects.

**Blood Sampling and Laboratory Analyses**

After an overnight fast, plasma samples were taken and stored at −70°C until analyzed. Nutritional status was recorded on the same occasion using subjective global assessment (SGA) (13,14) and each patient was given a score reflecting the nutritional status: 1, normal; 2 to 4; mild, moderate, and severe malnutrition, respectively. In the ESRD patients, GFR was estimated by the mean of urea and creatinine clearances (n = 159), whereas the GFR was evaluated by iothexol clearance in the elderly subjects. Determinations of serum albumin (bromcresol purple), C-reactive protein (CRP) (turbidometry), hemo-globin A1c% (HbA1c%), serum fibrinogen, and serum creatinine as well as urinary excretion of creatinine and urea were performed by routine procedures in the Department of Clinical Chemistry, Hud-dinge Hospital. The detection limit of CRP was 10 mg/L, and all values < 10 mg/L were in the statistical evaluation treated as 9 mg/L. High-sensitivity CRP (hsCRP) levels were available in 145 of the patients and was measured by the nephelometry method. IL-6 was measured by a photometric enzyme-linked immunosorbent assay (ELISA) obtained from Boehringer Mannheim (Mannheim, Germany). Soluble vascular cellular adhesion molecule-1 (sVCAM-1) was determined by commercially available ELISA kit (R&D Systems Europe Ltd, Abingdon, UK).

**Pentosidine Measurement**

Reverse-phase HPLC was chosen for the determination of the plasma pentosidine content. Although recent studies using specific ELISA for plasma pentosidine have shown results comparable with those obtained with HPLC assay, the latter is a more accurate and acceptable method for measuring the total pentosidine content because ELISA methods may not recognize pentosidine on the interior of proteins. Furthermore, ELISA methods require chemical hydrolysis and enzymatic digestion of protein, which may alter the epitope recognized by the antibodies. In this study, HPLC was used as described originally by Odetti et al. (7) and modified by Miyata et al. (15). Briefly, 50 µl of plasma were lyophilized and then hydrolyzed by 50 µl of 6 N HCl at 110°C under nitrogen atmosphere for 16 h, subsequently neutralized with 100 µl of 5 N NaOH and 200 µl of 0.5 M phosphate buffer (pH 7.4), and then filtered through a 0.45-µm Millipore filter and diluted 20-fold with phosphate-buffered saline (PBS). Filtered samples (50 µl) were then injected into C18 reverse-phase analytical column (218TP104, Vydac; Separation Group, Hesperia, CA) using online fluorescence detector at excitation/emission wavelengths of 335/385 nm. A linear solvent gradient was used as described by Wilker et al. (16), in which solvent A was 0.01 M
heptfluorobutyric acid (HFBA) in water and solvent B was 60% acetonitrile + 40% H2O + 0.01 M HFBA. The elution profile was as follows: 0 to 3 min, 0% B; 3 to 20 min, 0 to 30% B; 20 to 25 min, 30% B; 25 to 35 min, 30 to 100% B; 36 to 45 min, 0% B linear gradient. The flow rate was maintained at 1 ml through the chromatographic run. Synthetic pentosidine was used for calculation (kindly provided by Drs. David R. Sell and Vincent M. Monnier, Case Western Reserve University, Cleveland, Ohio, and by Dr. Toshio Miyata, School of Medicine, Tokai University, Japan). Because plasma total pentosidine is mainly present as protein bound and albumin is the only protein linking pentosidine (4), and because free pentosidine represents 3 to 4% of total circulating pentosidine (17), the plasma total pentosidine concentrations in pmol/L were, therefore, corrected for serum albumin and expressed as the plasma pentosidine content in pmol/mg of albumin (18).

Follow-Up Study

Survival was determined after a median follow-up period of 43 ± 2 mo (range, 1 to 96 mo), and the effects of variables predicting death were determined by the Cox regression method and the relative risk of death was calculated. Survival was measured from the day of examination until death (n = 54) or censoring for transplantation (n = 95), which was made at the end of the follow-up (November 15, 2002).

Statistical Analyses

Values are presented as mean ± SEM or medians (with range) with P < 0.05 taken to indicate a statistical significance. Comparison between the two groups was performed using nonparametric Mann-Whitney U test. Comparisons between two groups for nominal variables were made by Fisher exact test. Correlations were performed by Spearman Rank analysis. Survival analysis was made by the Kaplan-Meier and Cox regression analysis.

Results

The clinical characteristics of the patients close to the start of RRT are given in Table 1. The median plasma pentosidine levels were increased sevenfold in the ESRD patients (median, 29 pmol/mg albumin; range, 8 to 136 pmol/mg albumin) compared with the median level in elderly controls (median, 4 pmol/mg albumin; range, 2 to 8 pmol/mg albumin), respectively. All patients had a plasma pentosidine content above the 95th percentile of the levels in the elderly controls. There were no significant differences in the medians of the plasma pentosidine content between male and female both in the ESRD patients (29 [8 to 136] versus 27 [8 to 102] pmol/mg albumin, respectively) and the elderly subjects (4 [2 to 7] versus 4 [2 to 8] pmol/mg albumin, respectively). The plasma pentosidine content in ESRD patients and elderly controls were significantly correlated with both GFR (P < 0.01 and P < 0.05, respectively; Figure 1) and age (P < 0.001 and P < 0.05, respectively; Figure 2).

Patients with DM had a median plasma pentosidine (median, 32 pmol/mg albumin; range, 10 to 85 pmol/ml) comparable with the median in nondiabetic patients (median, 29 pmol/mg albumin; range, 8 to 136 pmol/mg albumin), and no association was found between plasma pentosidine and HbA1c%.

Seventy-three (38%) patients with signs of inflammation (CRP ≥ 10 mg/L) had a significantly (P < 0.001) higher plasma pentosidine content (median, 37 pmol/ml; range, 9 to 123 pmol/mg albumin) than 118 noninflamed patients (median, 24 pmol/mg albumin; range, 8 to 136 pmol/mg albumin). Moreover, the patients who had IL-6 levels above the median (n = 79) had significantly higher (P < 0.0001) plasma pentosidine content (median, 36 pmol/mg albumin; range, 18 to 123 pmol/mg albumin) than the patients who had IL-6 levels equal or below the median (n = 90; median, 23 pmol/mg albumin; range, 12 to 136 pmol/mg albumin).

A significant positive correlation was found between plasma pentosidine and CRP (Rho = 0.28; P < 0.0001). This relationship became somewhat stronger (Rho = 0.35; P < 0.0001) when hsCRP was correlated with plasma pentosidine in a subset of 145 patients. Moreover, the plasma pentosidine content was positively correlated with fibrinogen (Rho = 0.23; P < 0.01), IL-6 (Rho = 0.22; P < 0.01), and sVCAM (Rho = 0.38; P < 0.001) levels (Figure 3).

Sixty-three (33%) malnourished patients (SGA 2 to 4) had a significantly (P < 0.05) higher plasma pentosidine content (median, 39 pmol/mg albumin; range, 8 to 102 pmol/mg albumin) than 117 well-nourished patients (median, 27 pmol/mg albumin; range, 8 to 136 pmol/mg albumin). Sixty-two (32%) patients with CVD_clin showed no significant difference (P = 0.08) in the median plasma pentosidine content (median, 32 pmol/mg albumin; range, 12 to 85 pmol/mg albumin) compared with 129 patients without CVD_clin (median, 27 pmol/mg albumin; range, 8 to 136 pmol/mg albumin).

At follow-up, after a mean observation period of 43 ± 2 mo, 54 (28%) of the patients had died. The basal clinical and biochemical characteristics of survivors and nonsurvivors, respectively, are shown in Table 2. The prevalence of male gender did not differ between the two groups, whereas the difference in plasma pentosidine content (P = 0.04) between survivors (median, 39 pmol/mg albumin; range, 8 to 136 pmol/mg albumin) and nonsurvivors (median, 32 pmol/mg albumin; range, 8 to 136 pmol/mg albumin) was statistically significant.

<table>
<thead>
<tr>
<th>Table 1. Basal clinical and nutritional characteristics data before start of renal replacement therapy</th>
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</thead>
<tbody>
<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Prevalence male gender (%)</td>
</tr>
<tr>
<td>Prevalence of diabetes mellitus (%)</td>
</tr>
<tr>
<td>Prevalence of malnutrition (SGA &gt;1)</td>
</tr>
<tr>
<td>Prevalence of inflammation (CRP ≥ 0 mg/L) (%)</td>
</tr>
<tr>
<td>Prevalence of cardiovascular disease (%)</td>
</tr>
<tr>
<td>S-albumin (g/L)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
</tr>
<tr>
<td>S-creatinine (μmol/L)</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min)</td>
</tr>
<tr>
<td>Plasma pentosidine (pmol/ml)</td>
</tr>
<tr>
<td>Plasma pentosidine (pmol/mg albumin)</td>
</tr>
</tbody>
</table>

Mean ± SEM.

a n = 180.
b n = 159.
c median and range.
Prevalence of malnutrition, DM, inflammation, and CVD\textsubscript{clin} were significantly higher in the nonsurvivors. Moreover, as expected, nonsurvivors were older and had lower S-albumin, higher HbA1c% levels, and a higher median hsCRP level than survivors. The median plasma pentosidine level tended to be higher in the nonsurvivors than in the survivors, but this difference did not reach statistical significance ($P = 0.08$).

Univariate Cox-regression analysis (Table 3) shows that age, CVD\textsubscript{clin}, malnutrition, DM, S-albumin, and inflammation, but not pentosidine and gender, were associated with all-cause mortality. Furthermore, in a multivariate Cox-regression model including these variables, only age, CVD\textsubscript{clin}, malnutrition, and DM were independent predictors of mortality (Table 3). Since the multivariate analysis included two variables, which are potential markers of inflammation (CRP and pentosidine), that were correlated, it is possible that a colinear relationship between these variables may obscure the effect of each individually. Therefore, we also entered them sequentially in the model and found similar results (data not shown). Similarly, survival analysis by Kaplan-Meier, dividing patients into pentosidine quartiles, did not show any different survival rate between the four quartiles (Figure 4). Moreover, analyses of the data after censoring for transplantation did not change the results of the survival analyses. Also, Kaplan-Meier analysis did not show any association between the plasma pentosidine content (quartiles) and deaths attributed to CVD\textsubscript{clin} (data not shown).

**Discussion**

This study shows that the plasma pentosidine level is related to low residual renal function and high age but also to both inflammation and malnutrition in ESRD patients close to the start of dialysis therapy. However, plasma pentosidine level did not predict all-cause mortality in this patient group.
Pentosidine in Relation to Renal Function, Age, and Diabetes

AGE are formed during the Maillard reaction by nonenzymatic glycation and oxidation of proteins and accumulate in the course of aging and at accelerated rates in diabetes and uremia. It has been suggested that AGE contribute to long-term complications of ESRD, such as accelerated atherosclerosis and dialysis-related amyloidosis (19). In the current study, the inverse relationship between the plasma pentosidine content and GFR in both the elderly subjects and in the ESRD patients (Figure 1) are in accordance with previous studies demonstrating that the marked increase of the plasma pentosidine content is related to a decline in renal function. The well-known influence of aging on AGE levels is supported by the findings that age was related to the plasma pentosidine content in the elderly subjects and in the ESRD patients (Figure 2), suggesting that age is a factor determining AGE also in ESRD patients at the start of dialysis therapy. In patients with DM, increased AGE levels have been ascribed to high plasma glucose concentrations and a strong correlation exists between AGE and fructoselysine, a marker of plasma glucose concentration (1). The finding in the present study, which shows that the levels of pentosidine are not significantly different in ESRD patients with and without DM, is in accordance with previous studies.

Table 2. Clinical and nutritional parameters in survivors and nonsurvivors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Survivors (n = 37)</th>
<th>Nonsurvivors (n = 54)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation period (mo)</td>
<td>47 ± 2</td>
<td>31 ± 3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>50 ± 1</td>
<td>60 ± 1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6 ± 0.4</td>
<td>25.2 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Prevalence male gender (%)</td>
<td>61</td>
<td>69</td>
<td>NS</td>
</tr>
<tr>
<td>Prevalence malnutrition (SGA &gt; 1) (%)a</td>
<td>25</td>
<td>61</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Prevalence diabetes mellitus (%)</td>
<td>19</td>
<td>50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Prevalence cardiovascular disease (%)</td>
<td>20</td>
<td>61</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GFR (ml/min)b</td>
<td>6.8 ± 0.2</td>
<td>6.6 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>hsCRP (mg/L)cde</td>
<td>4.9 (0.2 to 89.0)</td>
<td>15.0 (0.3 to 163.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.3 ± 0.1</td>
<td>6.2 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S-albumin (g/L)</td>
<td>34.1 ± 0.5</td>
<td>31.0 ± 0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plasma pentosidine (pmol/mg albumin)d</td>
<td>27 (8 to 136)</td>
<td>36 (9 to 94)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma pentosidine (pmol/ml)d</td>
<td>905 (262 to 4698)</td>
<td>1054 (322 to 3964)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean ± SEM.

a n = 180.
b n = 159.
c n = 145.
d median and range.

Table 3. Univariate and multivariate analyses of the effect of age, gender, co-morbidity (diabetes mellitus, CVD, malnutrition, and inflammation), pentosidine, and s-albumin on the survival (risk ratio and 95% confidence interval by Cox regression model)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Relative Mortality Ratio</th>
<th>95% Confidence Interval</th>
<th>Probability</th>
<th>Relative Mortality Ratio</th>
<th>95% Confidence Interval</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per yr)</td>
<td>1.08</td>
<td>1.05 to 1.12</td>
<td>&lt;0.001</td>
<td>1.05</td>
<td>1.02 to 1.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.63</td>
<td>1.49 to 4.55</td>
<td>&lt;0.001</td>
<td>2.63</td>
<td>1.45 to 4.76</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Malnutrition (SGA &gt;1)a</td>
<td>3.65</td>
<td>2.07 to 6.43</td>
<td>&lt;0.001</td>
<td>2.29</td>
<td>1.20 to 4.36</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>4.76</td>
<td>2.77 to 9.09</td>
<td>&lt;0.001</td>
<td>2.27</td>
<td>1.23 to 4.34</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum albumin (per g/L increase)</td>
<td>0.91</td>
<td>0.87 to 0.95</td>
<td>&lt;0.001</td>
<td>0.95</td>
<td>0.90 to 1.00</td>
<td>NS</td>
</tr>
<tr>
<td>Inflammation (CRP &gt;10 mg/l)</td>
<td>2.63</td>
<td>1.49 to 5.54</td>
<td>&lt;0.01</td>
<td>1.03</td>
<td>0.51 to 2.08</td>
<td>NS</td>
</tr>
<tr>
<td>Male gender</td>
<td>1.33</td>
<td>0.75 to 2.38</td>
<td>NS</td>
<td>1.15</td>
<td>0.62 to 1.32</td>
<td>NS</td>
</tr>
<tr>
<td>Pentosidine (quartile 1 versus quartile 4)</td>
<td>0.93</td>
<td>0.42 to 2.04</td>
<td>NS</td>
<td>1.22</td>
<td>0.48 to 3.11</td>
<td>NS</td>
</tr>
<tr>
<td>Pentosidine (quartile 2 versus quartile 4)</td>
<td>0.97</td>
<td>0.45 to 2.09</td>
<td>NS</td>
<td>1.67</td>
<td>0.69 to 4.03</td>
<td>NS</td>
</tr>
<tr>
<td>Pentosidine (quartile 3 versus quartile 4)</td>
<td>1.26</td>
<td>0.62 to 2.56</td>
<td>NS</td>
<td>1.82</td>
<td>0.83 to 3.96</td>
<td>NS</td>
</tr>
</tbody>
</table>

| a n = 180. χ² = 75.82 for 60 mo.

Pentosidine in Relation to Renal Function, Age, and Diabetes

AGE are formed during the Maillard reaction by nonenzymatic glycation and oxidation of proteins and accumulate in the course of aging and at accelerated rates in diabetes and uremia. It has been suggested that AGE contribute to long-term complications of ESRD, such as accelerated atherosclerosis and dialysis-related amyloidosis (19). In the current study, the inverse relationship between the plasma pentosidine content and GFR in both the elderly subjects and in the ESRD patients (Figure 1) are in accordance with previous studies demonstrating that the marked increase of the plasma pentosidine content is related to a decline in renal function. The well-known influence of aging on AGE levels is supported by the findings that age was related to the plasma pentosidine content in the elderly subjects and in the ESRD patients (Figure 2), suggesting that age is a factor determining AGE also in ESRD patients at the start of dialysis therapy. In patients with DM, increased AGE levels have been ascribed to high plasma glucose concentrations and a strong correlation exists between AGE and fructoselysine, a marker of plasma glucose concentration (1). The finding in the present study, which shows that the levels of pentosidine are not significantly different in ESRD patients with and without DM, is in accordance with previous studies.
Moreover, we found no correlation between the plasma pentosidine content and HbA1c %, which suggests that the effect of DM on the plasma pentosidine content in ESRD patients is small compared with the substantial increase due to uremia per se. Our findings are also supported by the previously reported lack of relationship between fructoselysine and CML in ESRD patients (4).

**Pentosidine and Inflammation**

To determine the influence of inflammation in ESRD patients on plasma pentosidine content, we classified the patients into two groups on the basis of CRP levels. In the inflamed (CRP > 10 mg/L) patients, the plasma pentosidine was significantly higher than in the noninflamed patients, and CRP levels were significantly correlated with plasma pentosidine contents. This relationship became stronger when the relationship to hsCRP was determined, and it is further supported by the findings that the pentosidine levels were significantly correlated with these other markers of inflammation, IL-6, serum fibrinogen, and sVCAM-1 levels. The reason for the accumulation of AGE in uremia is only partly understood although it can be attributed mainly to the reduction in renal function. However, concomitantly with the accumulation of AGE, ESRD is characterized also by an increase in circulating levels of pro-inflammatory cytokines and acute phase proteins. It has been reported that inflammation may have a role in the production of AGE by generation of reactive aldehydes, which play a critical role in the formation of AGE and oxidative tissue damage (20). On the other hand, AGE may activate mononuclear cells, thus directly provoking an inflammatory response (21). Kislinger et al. (22) have recently shown that CML is a ligand for AGE receptors (RAGE) and modulates the nuclear translocation of NF-κB, which is the main cell signal for the activation of a range of inflammatory genes, including RAGE itself. Also, it has been reported that AGE stimulate a number of pro-inflammatory cytokines (23) and in HD patients the presence of purified AGE-modified β2-microglobulin also stimulates synthesis and secretion of IL-6, TNF-α, and IL-1β (24). Moreover, a positive correlation between CRP and plasma pentosidine has previously been reported by several groups in patients with rheumatoid arthritis (18,25,26). Miyata et al. (18) have shown that CRP is an independent predictor of plasma pentosidine levels in patients with rheumatoid arthritis. Furthermore, a relationship between pentosidine and inflammation evaluated by advanced oxidized protein products (AOPP), a mediator of inflammation and monocyte activation, and neopterin, a monocyte activation marker, have been previously reported in patients with different stages of chronic kidney disease (27,28). In this study, this relationship is further confirmed by the positive correlations between the circulating pentosidine content and common markers of inflammation, such as CRP and IL-6, suggesting that the high prevalence of inflammation and the accumulation of AGE in uremic patients are related.

**Pentosidine and Malnutrition**

At present, it remains unknown whether AGE accumulation in ESRD plays a role in the high prevalence of malnutrition or whether malnutrition has an influence on increases of AGE. AGE are known to form in certain processed foods (29). Koschinsky et al. (30) have suggested that dietary AGE might contribute significantly to the total AGE in the body. Moreover, experimental studies in support of a role for food-derived AGE in tissue pathology have begun to emerge (31–33). Nevertheless, although likely, it is not known whether food intake contributes significantly to accumulation of AGE in uremia. Miyata et al. (4) have suggested that dietary pentosidine may represent a possible origin of circulating free pentosidine. It has been recently reported (34) that restriction of dietary AGE intake may reduce the circulating AGE in uremic patients. To investigate a possible relationship between plasma pentosidine

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**Figure 3.** Relationship between plasma pentosidine content and soluble vascular cellular adhesion molecule-1 (sVCAM-1) in 74 ESRD patients close to start of dialysis treatment.

**Figure 4.** Kaplan-Meier plots of the survival rate of ESRD patients close to start of dialysis treatment during 5-yr follow-up with regard to all-cause mortality in relation to quartiles (Q1 < 20.28; Q2 20.29 to 28.71; Q3 28.72 to 43.94; Q4 > 43.94 pmol/mg albumin) of plasma pentosidine content. Differences between the groups were NS (χ² = 0.11; P = 0.74).
contents and nutritional status in ESRD patients, the patients were divided into two groups on the basis of SGA. The malnourished patients had significantly higher plasma pentosidine levels than the patients with normal nutritional status. These data thus suggest that malnutrition, which is strongly interrelated to inflammation and CVD in ESRD patients (12), might be involved in the process leading to accumulation of AGE. Hypoalbuminemia is a common feature observed in patients with inflammation and malnutrition, and both these conditions were characterized by high plasma pentosidine content in this study. Moreover, the formation of pentosidine and CML are known to be closely linked to oxidation (35). Himelfarb et al. (36) demonstrated that S-albumin in uremic patients is the main target of protein oxidation, which accounts for virtually all of the increased plasma protein carbonyl formation in such patients. Therefore, since pentosidine is a protein oxidation product, one possible explanation for these findings could be that pentosidine is a marker of the presence of oxidative stress mediated by malnutrition and inflammation.

**Pentosidine and Cardiovascular Disease**

Recent literature reports have proposed a link between AGE formation and development of CVD (19). In ESRD, however, there is still no study available showing that AGE are independent risk factors for CVD. Recently, Zoccali et al. (10) reported that plasma pentosidine levels were not related to severity of atherosclerosis, but to left ventricular hypertrophy in HD patients. In the current study, the pentosidine levels in ESRD patients were more than seven times higher, and all patients had higher levels than the elderly subjects, but no discrimination between the patients with and those without CVD \(_{\text{clin}}\). However, the small number of patients as well as the crude classification of CVD \(_{\text{clin}}\) may introduce a selection bias in this study, because the role of pentosidine is presumably in the process of formation of atheroma and a clinical definition of CVD clearly does not measure the atheromatous burden. Nevertheless, we found that the pentosidine content was strongly correlated with the levels of sVCAM-1 in a subset of the patient population (Figure 3). Soluble adhesion molecules are expressed on the surface of vascular endothelial cells in response to pro-inflammatory cytokines and may play an important role in the atherogenic process (37). Moreover, expression of several adhesion molecules was found on established atherosclerotic lesions (37,38) and elevated serum levels of soluble adhesion molecules have been demonstrated in patients with CVD (39–41). The plasma pentosidine content in this study was high in all the ESRD patients and even higher in the subset of the patients with inflammation/malnutrition and furthermore related to the sVCAM-1, suggesting a pathophysiological role of pentosidine in the atherogenic process or possibly that pentosidine is a marker of oxidative and inflammatory activity present in atheroma. Taken together, the fact that all the patients had elevated pentosidine levels may explain the lack of discrimination between pentosidine content in the patients with and without CVD \(_{\text{clin}}\). Consequently our findings do not exclude a role of carbonyl stress for the development of CVD in ESRD patients.

**Pentosidine in Relation to All-Cause Mortality**

The present study was also aimed to prospectively study the possible link between accumulation of AGE and all-cause mortality in ESRD patients. The pentosidine content tended to be higher in the nonsurvivors than in the survivors (Table 2), but this difference did not reach statistical significance (\(P = 0.08\)). There may be several reasons for this, such as the small sample size and insufficient power to detect a clinically important difference in plasma pentosidine content. However, the patients in all four quartiles of the study had a similar 5-yr survival rate (Figure 4). These findings basically accord with a recent study by Schwedler et al. (11), demonstrating that high levels of CML and total serum fluorescence AGE are not linked to mortality in HD patients. Although a relationship between various inflammatory markers and plasma pentosidine was found in the current study, these findings, unexpectedly, do not offer solid support for the hypothesis that AGE are linked to comorbidity and mortality by activating the acute phase response in ESRD patients. This may reinforce the concept that outcome in ESRD patients is affected by a multitude of factors, each exerting its effect without being necessarily responsible for the end point event. From these findings, it appears that circulating AGE may not be clinically meaningful parameters to use for outcome prediction in ESRD patients. Also, it has been argued that the circulating AGE may not represent total body AGE (11). In support of this assumption, a recent observation of a link between intracellular concentrations of AGE and risk of diabetic retinopathy has been reported (42).

**Limitations of the Study**

Several shortcomings of the present study should be considered. First, as we relied on a single determination of plasma pentosidine, we cannot take into account any variation of pentosidine content that may have occurred over time. Second, it is plausible that our findings are limited by the classification of CVD that included only patients with clinically significant disease, which may underestimate the real difference among pentosidine contents between patients with and without CVD. Third, a rather limited number of patients were studied, and more studies including larger numbers of patients are needed to confirm our findings. Finally, it should be pointed out that this is a post-hoc analysis, which may limit the value of the study.

In summary, it can be concluded from the present study that the plasma pentosidine content was high in all ESRD patients and that the higher plasma pentosidine content was linked to inflammation and malnutrition. In addition, both low residual renal function and age contribute to increased plasma pentosidine levels in ESRD patients. Overall these findings are consistent with the hypothesis that AGE are linked to co-morbidity by activating the acute phase response. However, surprisingly, the plasma pentosidine content was not found to be associated with all-cause mortality. Thus, accumulation of plasma pentosidine in ESRD is unlikely to be an appropriate marker to predict mortality.
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